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The manuscript should be typewritten on one side of the paper only, with wide margins and be double spaced throughout including titles, footnotes, literature citations and legends. Symbols, formulae and equations must be written clearly and with great care. Scientific names of genera and species are printed in italics and should be underlined in the typescript. Too many tables, graphs, etc. should be avoided. Each table should be typed on a separate sheet with its proper position marked in the text in pencil.

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JOURNAL

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J. Madras Univ., B. XXXI, No. 2, Pages 109-119, 1961.

Temperature variation in *Ocypoda macrocera**

BY

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(Received for publication on 14-4-61)

ABSTRACT

The temperature variation in *Ocypoda macrocera* was studied in relation to varying environmental conditions. The body temperature was lower than the aerial temperature when the humidity of the air was low, and high in saturated condition. The body surface exposed to air as well as muscular activity were found to influence the body temperature.

The body temperature of aquatic and terrestrial poikilotherms have been studied by many [Simpson (1908), Rogers & Lewis (1916); Baldwin (1925); Necheles (1924); Koidsumi (1935), Gunn (1935), Ramsay (1935), Mellanby (1932), Digby (1955), Edney (1951b), Parry (1951), Church (1960)]. However among terrestrial poikilotherms most of the work has been confined to insects.

Our knowledge of the body temperature of poikilotherms of tropical region is very limited. It was therefore felt that a study of the body temperature of the common intertidal shore crabs would be of some interest. The focus of interest of this paper is the nature, extent, and causes of the divergence in body temperature in relation to varying environmental conditions. A series of experiments were performed to record the body temperature and the results obtained in relation to different environmental factors are presented here.

Material and methods

Ocypoda macrocera, universally found in sea shores inhabiting the intertidal zone was used for the study. Ocypoda live in burrows dug out with great ease by the aid of their legs. When

* A summary of the dissertation submitted for the M.Sc. degree of the Madras University.

tide recedes they come out of the burrows in search of food and return at the remote sign of danger. Temperature measurements showed that there exists a difference of about 1°C. between the temperature at the entrance and the terminus of the burrow. Crabs were collected in the beach opposite to the University Zoology Research Laboratory, Madras, and were maintained in moist sand partly covered.

Temperature measurements were made with the help of a thermocouple (Hartmann & Braun thermocouple). The junctions were made up of iron and constantan wire, an alloy of copper and nickel. In order to keep the reference temperature constant, the opposing couple was inserted into a thermoflask filled with water at room temperature while the other junction was in the form of a needle so that it could be inserted into the body of the crab. The thermocouple was connected to a calibrated sensitive galvanometer and the temperature was directly read from the dial provided.

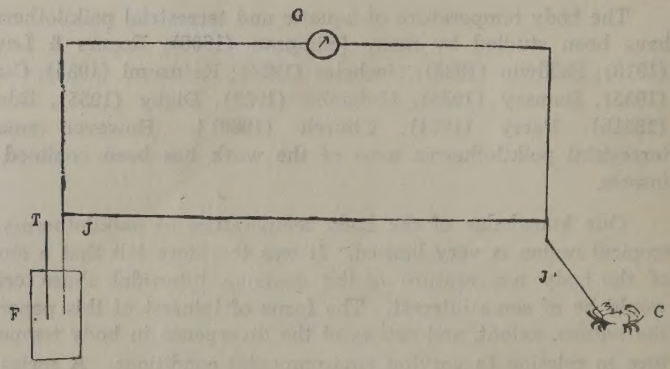

FIG. 1. To illustrate the arrangements of thermocouple for measuring the body temperature.

Key to lettering:— C-Crab; F-Thermoflask; J & J'-Thermo junctions; G-Galvanometer; T-Thermometer.

Body temperature of crabs was taken from the leg joint of chelicerae since it did not injure the animal. Temperature measurements made on the male and the female crabs showed that the sex does not affect the body temperature considerably. Hence both sexes were indiscriminately used in all the experiments. Humidity was measured with a small hygrometer (*'Edney'* Dial thermometer).

Observations

Preliminary observation:—

In summer (July & August) the body temperature of crabs varied from 28° to 32°C. when aerial temperature ranged from 32° to 34°C. Thus a difference of about 3° to 4°C. was observed between body and aerial temperature. But during monsoon period the body temperature was never below the aerial temperature, when the humidity was considerably high.

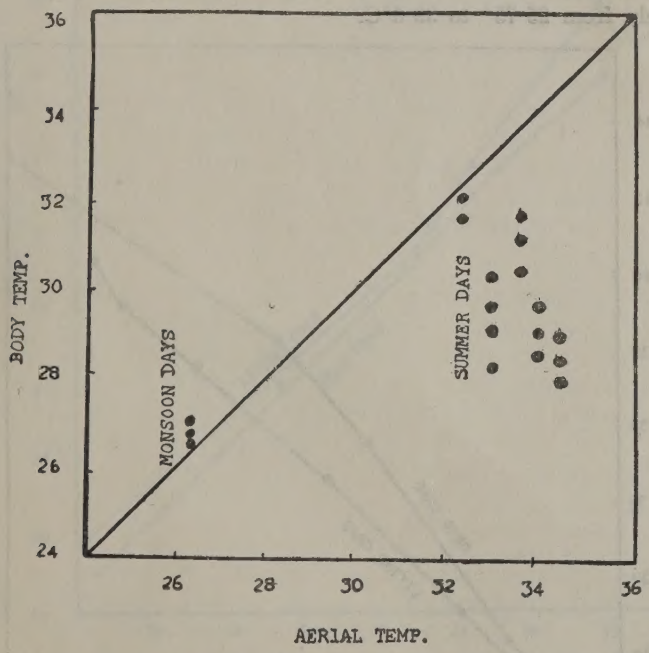

FIG. 2. To illustrate the difference between aerial temp. and body temp. a 'hypothetical isothermic line' is indicated in the graph and actual body temperatures have been plotted against aerial temp.

Body temperature and changes in environmental conditions:—

The environmental conditions are known to have a profound influence on the body temperature. Hence an attempt was made to bring about some changes in the environmental conditions and study their effect on body temperature of crabs. A few crabs were accordingly removed from the moist sand in which they were

having more or less the same environmental conditions and were introduced into beakers and were exposed to air for a few hours. In all the cases, there was an increase in the body temperature which ranged from 2° to 4°C. (28·4°—32·2°C.).

Further, the effect of sudden rise as well as fall in environmental condition and its effect on the body temperature was studied in detail. When the aerial temperature was raised artificially from 25·7° to 40·5°C. by adding hot water to the bottom of the container of the crab, the body temperature also followed closely from 25·75° to 39·6°C.

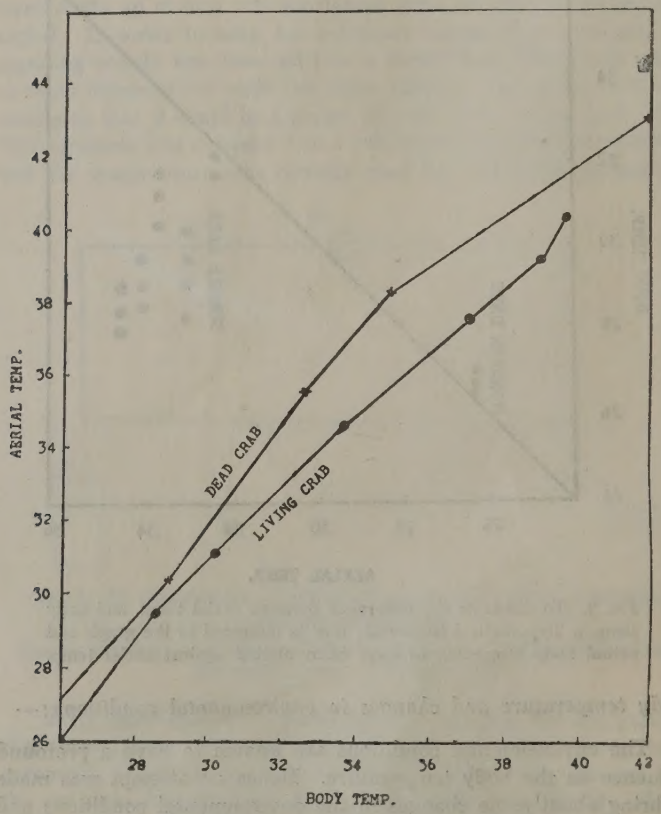

Fig. 3. To illustrate the relation between the body temp. and the aerial temp. when the environmental temp. was raised artificially. Temperature behaviour of dead crabs also shown.

Similarly by lowering the aerial temperature from 39·5° to 19·75°C., the body temperature also fell with aerial temperature from 34° to 18°C.

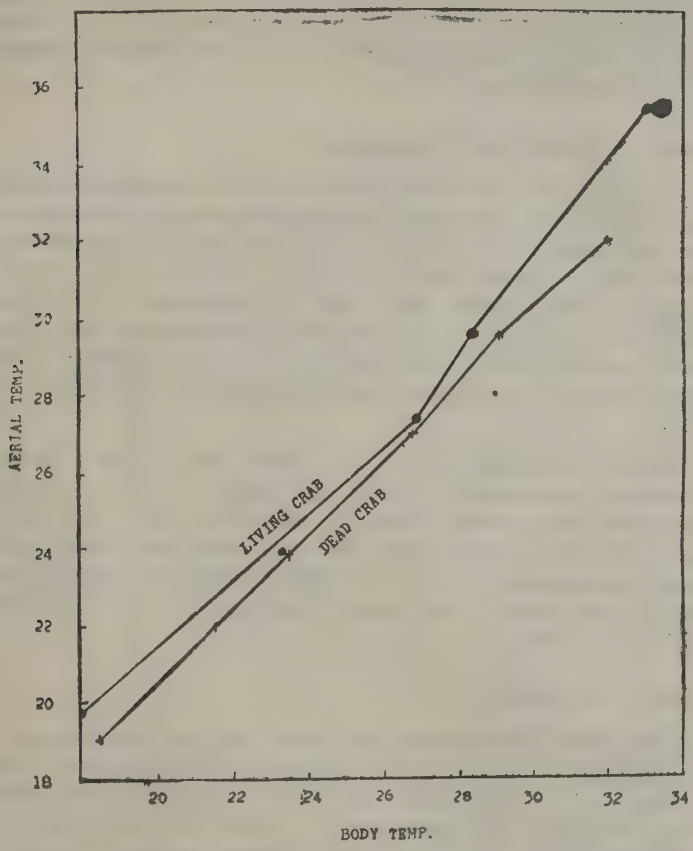

Fig. 4. To illustrate the relation between aerial temp. and body temp. when lowered.

Biological regulations: —

Ability of poikilotherms for regulating body temperature has attracted the attention of many (Buxton, 1924; Necheles, 1924; Mellanby, 1932; Ramsay, 1935; Edney, 1951). An attempt was made in this line too on crabs. The same experiments on environmental changes (Figs. 3 & 4) conducted on living crabs were

repeated with dead crabs. No notable difference was found between living and non-living forms. When the aerial temperature was raised from 27° to 43°C., the body temperature also rose from 26° to 42°C. Similarly when the aerial temperature was lowered from 38·4° to 19°C., the body temperature also fell from 38·4° to 18·5°C. Hence an active capacity for regulation seems to be wanting (Figs. 3 & 4).

Body size, Weight and Temperature : —

Since it was known that body size has a significant role on body temperature, the relation between the body size, weight and body temperature was studied in a few forms. According to Gunn (1942), the surface-weight ratio has little bearing on body temperature at a steady state. But as environmental conditions change a large animal would change its temperature more slowly than a small one. At any rate small animals generally have a higher rate of heat production per gram weight than large animals.

In the data collected on a few crabs, more or less a similar observation was made. A small crab weighing 7·3 gms. had a higher body temperature (31·65°C.) than a few others (31·25°C.) double its weight (16·2 gms.). In the same way a small crab with a surface area (1·7 × 1 sq. cm.) had a higher temperature (29°C.) than most of the larger specimens (28°C.) measuring 3·5 × 3·7 sq. cms.

Effect of evaporation : —

The above experiments have shown that the surface area is an important factor and hence its role on body temperature was studied. In a few crabs the body surface-area exposed, was reduced by plugging the gills and mouth by plasticene, leaving the legs alone free. They were then kept in a warm container along with a few other normal forms. An increase ranging about 2° to 5°C. was noticed in the crabs whose gills and mouth was plugged whereas in others the maximum rise was only 1·5°C. (29·6°—31·1°C.).

Humidity and Body temperature : —

Among the factors studied humidity appears to influence the body temperature considerably. So the interrelation between

the two were studied in detail. Body temperature of crabs were noted at different humidities having kept them inside a desiccator containing $CaCl_2$ and H_2SO_4. Measurements showed that there was a fall in both humidity (72—60) as well as in body temperature (27°—24°C.) within a period of about three hours.

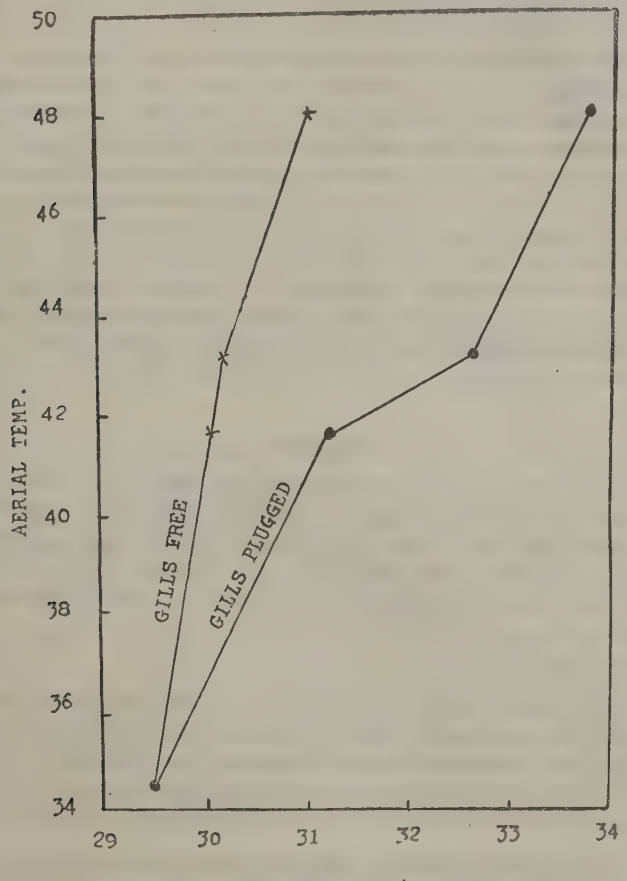

FIG. 5. To illustrate the relation between the body temp. of crabs whose gills plugged and free.

Southward (1958) has shown in barnacles and topshells that body temperature is high when humidity is high. That is why on rainy days, during monsoon period the body temperature was found to be even above the aerial temperature (Vide Fig. 2).

Muscular activity : —

It is known that muscular activity influences the body temperature. An attempt was made to know the inter relation between these two. In *Calliphora,* Digby (1955) noticed a rise of upto 2°C and it was followed by a slow drop of 1°C. after flight had continued for some minutes.

A similar behaviour was noticed in the body temperature of crabs when they were forced to be active. At first a rotating mill was set up and the crab was tied to it. But since the crab did not move along the mill another easy and simple device was arranged. A few crabs were suspended from a stand and they went on wriggling in an attempt to free themselves with consequent increased muscular activity. An increase of about 1°C. was noticed in one form (30°—31°C.). In all, the rise was gradual and it was followed by a slow drop. It must be stated that this much of rise in temperature is significant, because a great part of the total heat produced should have been lost by evaporation, conduction, radiation etc. in the absence of insulation.

Discussion

Temperature measurements in crabs during summer and monsoon period showed that the body temperature is higher than the aerial temperature during the latter period. Necheles (1924) working on *Blatta orientalis* stated that "Above about 25°C. the body temperature depended on the humidity of the air, so that in moist air it was slightly above and in dry air below the temperature of the air."

In a tropical region like Madras, the temperature as well as humidity is considerably high throughout the year. Even during summer the humidity does not fall below about 50% and the saturation of the air in monsoon period is above about 90%. Hence the influence of the various factors appears to be so complex.

The influence of environment on body temperature was found to be significant on crabs. The experiments conducted to study the relation showed that the body temperature followed closely the aerial temperature when environmental temperature was raised as well as lowered artificially (Figs. 3 & 4).

The temperature of the body could be raised with the reduction of the exposed body surface by plugging the gills and mouth,

thus minimising the rate of evaporation. Gunn (1942) reviewing the evaporation in insects has remarked "Evaporation of water may cool the animals by several degrees below the air around it especially if the air is dry." Evaporation is found to be limited when the surrounding air is saturated with moisture. Hence body temperature of crabs was found to be high when the humidity of the air was high. That was why during the rainy season the body temperature was found to be above the aerial temperature. According to Necheles (1924), Mellanby (1932), Southward (1958) and Edney (1951b) a considerable depression in body temperature was seen only in dry air. A decrease in humidity is found to be correlated with loss of weight and fall in body temperature as might be expected.

The experiments on mascular activities showed that there was an increase of about 1°C. But this increase in temperature did not last long. Many authors who have worked on insects [Dotterweich, (1928); Kalmus, (1929); Church, (1960)] also have observed considerable increase in body temperature in relation to muscular activity.

On studying the relation between size, weight and body temperature large and massive specimens were found to have low temperature than small specimens. Gunn (*loc. cit.*) in his review has made a similar observation. "As far as evaporation is concerned large animals can afford to keep cool by evaporation, small ones cannot, for they dry up too quickly."

As far as the body temperature of crabs was concerned, no active regulatory capacity was observed. For the experiments conducted on dead crabs also showed more or less the same readings as in the living forms. In *Ligia* Edney (1951a) observed no difference between living and nonliving forms. Ramsay (1935) working on cockroach remarked "It hardly seems justifiable to think of any regulation."

The factors that have been dealt with in the course of the study do not have the same importance on body temperature and the influence of any single factor cannot be considered as a criterion for evaluating the nature of body temperature. For the interrelation that exists between the different factors are so complex that it is impossible to mention one without the interference of others.

Acknowledgements

I am much indebted to Dr. S. Krishnaswamy, Reader, Zoology Research Laboratory, for suggesting this problem, guidance and encouragement. I am thankful to Dr. C. P. Gnanamuthu, Director, for giving me all the facilities and evincing great interest in my work.

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J. Madras Univ., B. XXXI, No. 2, Pages 121-122, 1961.

A note on *Azygophleps scalaris* Fabr. as a Pest of *Sesbania speciosa*

BY

S. Venugopal and K. R. Nagaraja Rao
Agricultural College, Coimbatore

(Received for publication on 23-4-61)

Introduction

Among the green manure crops, *Sesbania speciosa* occupies a very important place in the Madras State and is grown extensively for manuring paddy fields. Three important pests have so far been recorded on this crop, viz., *Prodenia litura,* F., *Empoasca* sp. and *Hyposidra successaria,* Wlk. Very recently a serious attack of the stem borer *Azygophleps scalaris* (Family—*Zeuzeridue*) was noted for the first time at Coimbatore. Fletcher (1913) has made mention of this pest as damaging *Sesbania aculeata* and *Sesbania aegyptiaca.* Lefroy (1909) and Ramakrishna Iyer (1940) have noted the occurrence of this on *Sesbania grandiflora* as well. A brief account of this stem-borer as observed at Coimbatore is furnished below.

Life-history : —

The female moth lays about 1,500 to 1,800 eggs in a mass cemented together by sticky secretion in-between two folded leaflets. The egg is pale yellow in colour and oval in outline. The caterpillars hatch out in 5 to 6 days and gradually descend down. By the action of wind they get distributed to the adjoining plants. They usually attack the growing point and reach the main stem in due course, where the rest of their life cycle is passed. The newly hatched caterpillar is 1¼ m.m. long with a black head and greyish body, studded with pinkish warts. The full grown caterpillar is 60 to 75 m.m. long with slight transverse humps on the back of the body segments. The body is smooth, creamy white in colour, with inconspicuous setae. The head is very small, motile, retractile and partly concealed by the overlapping large prothorax. Both head and prothorax are coloured brownish black with toothed black mandibles. Ventral-region of the mouth parts

are pale white with chitinised brown areas. Vertex is broad, open and ad-frontal sutures extend to the vertex. The body shows numerous irregular wrinkles with blunt projections mostly located on the mid-dorsal lines on segments 2 to 7. The bulbous humps seem to pulsate or to make heart beat like movements. The anal plate is slightly chitinised and possesses small setae. The prolegs are stout, stumpy fleshy processes with thin planta and bearing crochets in an elliptical fashion. The hooks are uniform and in a single row. The caterpillar bores down the centre of the stem, and the tunnels thus caused are filled with frass which is occasionally ejected through holes made on the side walls of the stem. The larva makes an exit hole when full grown, through the outer portion of the stem and transforms into an elongated blunt reddish brown pupa inside the stem itself (Plate V). The larval period ranges from 60 to 80 days. The pupa has transverse rings of small hooked spines which help it to move up and down the gallery. The pupal period ranges from 14 to 16 days. The total life cycle of this pest is about 79 to 102 days. It is seasonal in occurrence and noted from July to November, and from January to April. The adult is a big white and brown spotted leopard moth.

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Sesbania speciosa stem showing the presence of grown up caterpillar and pupa.

J. Madras Univ., B. XXXI, No. 2, Pages 123-125, 1961.

Preparation of pure Zinc Dimethyl*

BY

R. Ganesan†

(Received for publication on 29-4-1961)

SUMMARY

The preparation of a pure sample of zinc dimethyl is described.

Introduction

Compounds having the general formula MR_x, where M represents a metal atom and R an alkyl radical, are called metal alkyls. They are very reactive, and some of them, such as zinc dimethyl and aluminium trimethyl, are spontaneously inflammable in air or oxygen. This paper deals with the preparation of a pure sample of zinc dimethyl for a kinetic study.

The method described by Renshaw and Greenlaw (1920) was followed. In this method methyl iodide is heated with a very active zinc-copper couple, in the presence of methyl acetate as catalyst. The reaction consists of two stages which may be represented as follows:

$$Zn + CH_3I \rightarrow CH_3ZnI$$
$$2CH_3ZnI \rightarrow Zn(CH_3)_2 + ZnI_2$$

Methyl zinc iodide is first formed as a solid at about 60°C. It then decomposes at a higher temperature (200°C) to give zinc dimethyl (b.p. 44°C). The preparation was carried out under an atmosphere of carbon dioxide.

Experimental

About 100 gm. of zinc (30 mesh) was successively treated with very dilute hydrochloric acid, water, alcohol and ether, and was finally dried. Copper in a finely divided state was obtained by

* Contribution from the Chemistry Department, Loyola College, Madras.

† Present address: Department of Physical Chemistry, University of Madras.

precipitating the metal from a warm 5% solution of copper sulphate, by the addition of zinc dust. 11 gm of copper and 99 gm of zinc were well mixed. The mixture was introduced (5 gm at a time) into a combustion tube and was uniformly heated in a stream of hydrogen until it turned into "lead-coloured" pellets of zinc-copper couple.

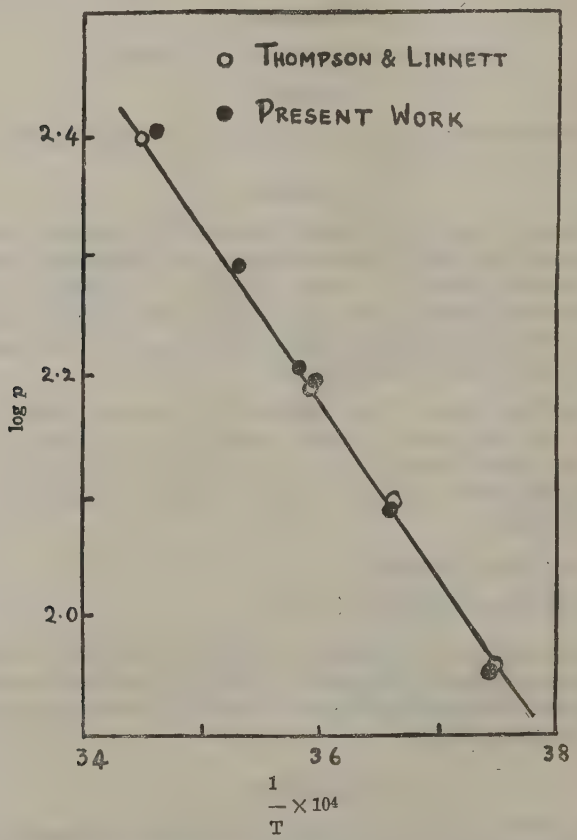

FIG. 1. Vapour pressure—Temperature plot for Zinc dimethyl

110 gm of the zinc-copper couple, 41 gm of methyl iodide (b.p. 42·5°C) and six drops of methyl acetate (b.p. 57·1°C) were placed in a two-necked flask which was provided with an inclined reflux condenser (Renshaw *et al* 1920) and an inlet for carbon dioxide.

The other parts of the refluxing apparatus were similar to those described by Renshaw and Greenlaw (1920). The flask was heated on a water bath (60°C) for twelve hours.

The flask and the condenser were then converted into a distillation apparatus (Renshaw *et al*, 1920) and heating was continued on the water bath. Thus the small amounts of unreacted methyl iodide and methyl acetate were removed by distillation. The boiling points of methyl iodide and zinc dimethyl are very close to each other. Hence the removal of the unreacted methyl iodide is very essential even at this stage, if a very pure sample of zinc dimethyl is ultimately aimed at. Probably, failure to carry out this operation was responsible for the slow separation of a white precipitate from the purified zinc dimethyl prepared earlier in this laboratory. The flask was finally heated in an oil bath (200°C). Methyl zinc iodide decomposed to give zinc dimethyl which was collected in a distillation flask containing about 1 gm of dry silver oxide (Badwin *et al*, 1947). The yield was about 10 cc.

The crude product was then fractionated in an atmosphere of carbon dioxide. The middle fraction (uncorrected b.p. 43·9°C) was collected. The metal alkyl was further purified by vacuum distillation in an all-glass apparatus. The vapour pressure (p) of the zinc dimethyl was roughly measured at a few temperatures in the range -6 to 16°C, and the values agreed with those obtained by Thompson and Linnett (1936). The plot of log p against the reciprocal of absolute temperature (T) is shown in Fig. 1.

Acknowledgement

The author wishes to express his thanks to Prof. L. M. Yeddanapalli for his helpful suggestions. Thanks are also due to Dr. V. J. Paul for his help in the experimental work, and to the University of Madras for the award of a research studentship.

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The Pyrolysis of Zinc Dimethyl*

BY

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SUMMARY

The thermal decomposition of zinc dimethyl vapour has been studied in a static system. At 305·5°C and in the concentration range 1 to 18 millimole/litre, the reaction rate decreases with increasing concentration. About two-thirds of the gaseous products is methane, the rest being C_2-hydrocarbons. When the reaction vessel is packed with glass tubes both the reaction rate and the ratio CH_4/C_2-hydrocarbons increase. This shows that the decomposition is heterogeneous and that a part of the methane is produced by the heterogeneous process.

Introduction

When metal alkyls undergo thermal decomposition in the vapour phase they yield free alkyl radicals (Paneth and Hofeditz, 1929) which are highly reactive. Kinetic studies of such processes give valuable information regarding the role of free radicals in chemical reactions. This paper deals with an investigation of the pyrolysis of zinc dimethyl in a static system.

Experimental

Materials

Zinc dimethyl was prepared by the action of methyl iodide on a very active zinc-copper couple (Renshaw and Greenlaw, 1920). It was purified by vacuum distillation *in situ* in the apparatus described below. Benzophenone (b.p. 305·5°C) and anthracene (b.p. 340°C) were the materials used in the vapour baths.

Apparatus

The experiments were carried out in an all-glass (Pyrex) gas-kinetic apparatus which is shown diagrammatically in Fig. 1. "Apie-

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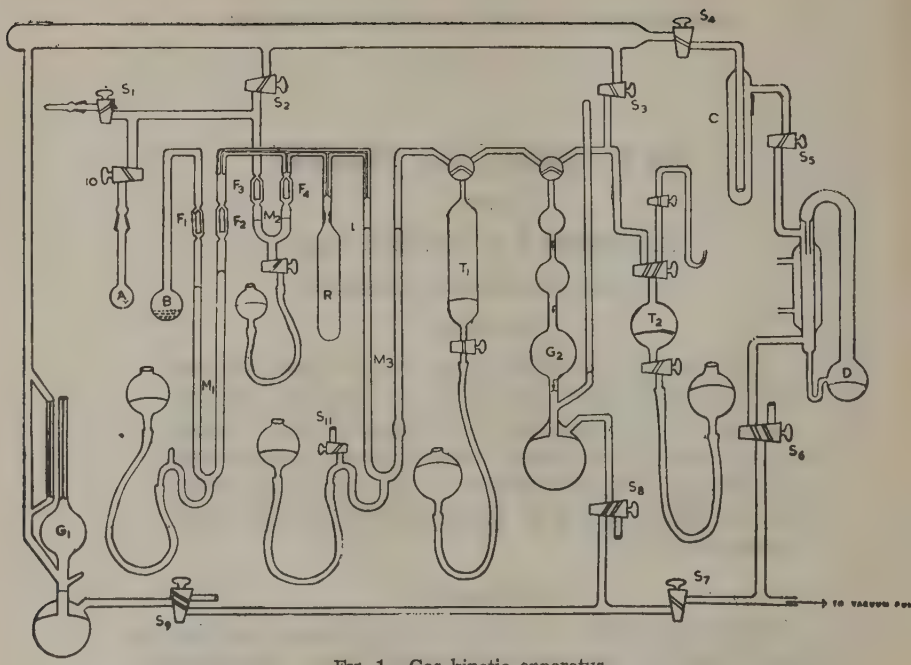

FIG. 1. Gas-kinetic apparatus.

B	Storage bulb containing zinc dimethyl
R	Reaction vessel
F_1, F_2, etc.	Ground-glass floats
M_1, M_2, M_3	Manometers
T_1, T_2	Töpler pumps
G_1, G_2	McLeod gauges
D	Mercury diffusion pump

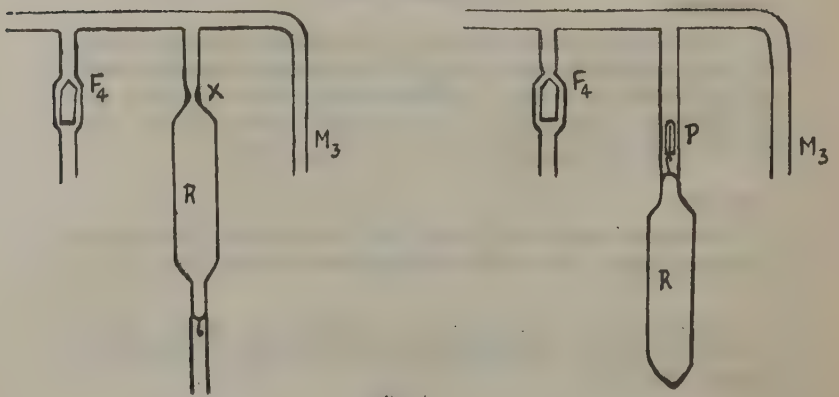

FIG. 2

zon-L" high vacuum grease was used for all the stop-cocks. Since zinc dimethyl reacts with grease, ground-glass floats F_1,F_2, etc., were incorporated at suitable places in the apparatus, so as to prevent the metal alkyl from coming into contact with the stop-cocks. The mercury diffusion pump (D) was backed by a rotary oil pump (not shown in the Fig. 1). All pressure measurements on the manometer (M_3) were made by a cathetometer. Mercury used in the manometers, gauges and Töpler pumps was periodically cleaned.

The reaction vessel (R) was not as shown in Fig. 1 but slightly of a different construction. It is shown in Fig. 2. The vessel was cylindrical in shape (length, 16 cm; diameter, 2·2 cm; area/volume, 1·8 cm^{-1}), and was provided with a capillary inner-seal at its lower end and with a constriction (X) on top (Fig. 2a). To study the effect of surface on the reaction, a few experiments were carried out in vessels packed with short pieces of Pyrex glass tubing of known surface area.

Procedure

Before each experiment the reaction vessel (of known volume) was cleaned with warm chromic acid solution and thoroughly rinsed with distilled water. It was then sealed on to the manometric system as shown in Fig. 2(a). After evacuating the vessel, the required quantity of zinc dimethyl vapour was admitted into it. The pressure of the metal alkyl was read on the manometer M_3. After immersing the vessel in liquid oxygen and thereby freezing the zinc dimethyl, the vessel was sealed off at the construction X. The decomposition of the metal alkyl was brought about by heating the vessel for an hour in an appropriate vapour bath (Cunningham and Taylor, 1938; Yeddanapalli *et al*, 1954). The vessel was then resealed to the manometric system as shown in Fig. 2b. After evacuating the dead space, connection between R and the manometric system was re-established by breaking the capillary inner-seal by means of the magnetically operated plunger P. The reaction vessel was surrounded by liquid oxygen, and the uncondensed gases were transferred by the Töpler pump (T_1) into the McLeod gauge G_2 in which their pressure was measured. A small quantity of this gas-mixture (methane and hydrogen) was transferred, if necessary, to a microgas-analysis apparatus (not shown in Fig. 1) by means of T_2. The liquid oxygen bath was then replaced by a dry-ice bath, and a second fraction of uncondensed gases (ethane and ethylene) was similarly removed from the

TABLE I

Thermal Decomposition of Zinc Dimethyl: Results in Packed and Unpacked Vessels

Reaction time, 60 minutes; Pressures are at O°C

Run	Area / Vol cm^{-1}	Temp °C	Initial $ZnMe_2$ mm.	Final $ZnMe_2$ mm.	Decomposition %	CH_4 mm.	C_2-hydrocarbons mm.	CH_4 / C_2-hydrocarbons
14	1·8	305·5	13·30	9·25	30·5	1·88	1·22	1·5
13	„	„	26·91	20·92	22·3	3·06	1·39	2·2
2	„	„	43·33	36·63	15·5	3·68	1·93	1·9
11	„	„	41·89	35·10	16·2	3·60	1·91	1·9
12	„	„	78·80	69·83	11·4	5·69	2·35	2·4
16	„	„	133·70	119·70	10·5	10·13	3·32	3·1
26	„	„	276·40	254·00	8·1	20·95	8·50	2·5
24	5·5	305·5	14·55	6·77	53·5	10·10	1·36	7·4
22	„	„	80·15	65·89	17·8	13·32	2·73	4·9
19	9·0	305·5	42·55	27·26	35·9	14·58	2·99	4·9
23	„	„	81·62	65·55	19·7	16·12	2·64	6·1
25	„	„	138·40	112·30	18·9	27·65	5·77	4·8
27	„	„	271·30	230·00	15·2	42·45	11·82	3·6
5	1·8	340·0	27·00	19·06	29·4	5·19	2·49	2·1
4	„	„	40·96	29·33	28·4	7·68	4·16	1·9
7	„	„	55·18	39·74	28·0	11·20	5·21	2·2

reaction vessel. The pressure of the undecomposed zinc dimethyl was read on M_3. The pressures of reactant and products were finally reduced to 0°C and the volume of the reaction vessel.

Results and Discussion

The results of runs at 305·5 and 340°C are given in Table 1. The runs at 340°C are in unpacked vessels. At 305·5°C results are quoted for packed vessels (area/volume, 5·5 and 9·0 cm^{-1}) also. The first gas fraction was analysed only in a few runs. It contained about 95% methane and only 5% hydrogen. The whole of this fraction is therefore represented as methane in all the runs in the table. The second fraction (C_2-hydro-carbons) was not analysed.

Unpacked vessels

Runs 2 and 11 illustrate that results are reproducible. In the three runs (with different initial concentrations of zinc dimethyl) at 340°C, the percentage decomposition is almost constant in a given reaction time. This is characteristic of a first-order reaction. At 305·5°C there is no such indication to show that the reaction is first order, except at high initial pressures (runs 12, 16 and 26). In fact the rate decreases with increasing pressure, an effect which seems to be due to the sensitivity of the reaction to surface. The amount of methane produced is nearly twice that of the C_2–hydrocarbons in all the runs.

Packed vessels

For a given initial pressure of zinc dimethyl, the reaction rate increases when the area/volume ratio is increased (e.g., runs 12, 22 and 23). This clearly shows that the reaction is at least partly heterogeneous. Another significant result in packed vessels is the marked increase in the amount of methane produced, without a proportional change in the amount of C_2-hydrocarbons (cf. runs 12, 22 and 23). The same result is clearly brought out, for all the runs, by the values of the ratio CH_4 C_2-hydrocarbons, given in the last column of the Table. The conclusion to be drawn from these observations is that methane is being produced by a process which is at least in part heterogeneous.

Solid products

In addition to the gaseous products, a dark brown deposit was formed on the walls of the reaction vessel. The deposit is consi-

dered to be carbon and a methylene polymer (Laurie *et al*, 1955; Cunningham *et al*, 1938; Yeddanapalli *et al*, 1954). As anticipated, zinc was also deposited in a finely divided state inside the vessel.

Probable reaction steps

It is known that the primary process in the decomposition of metal alkyls is the production of free alkyl radicals. Accordingly, the process for zinc dimethyl is

$$Zn(CH_3)_2 \rightarrow Zn + 2CH_3 \qquad (1)$$

The probable steps for the formation of methane and ethane are (Ganesan, 1961)

$$CH_3 + Zn(CH_3)_2 \rightarrow CH_4 + CH_3ZnCH_2 \qquad (2)$$

and

$$CH_3 + CH_3 \rightarrow C_2H_6. \qquad (3)$$

The present results do not, however, show whether reaction (2) is the only step producing methane both in the gas phase and on the surface. Further work, elucidating this and various other aspects of the problem, will be published elsewhere.

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The authors wish to thank Dr. V. J. Paul for assistance during the course of this work. One of the authors (R. G.) is thankful to the University of Madras for the award of a research studentship.

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Embryological Studies in the Hydrocharitaceae
1. *Blyxa octandra* Planch

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The earliest embryological literature on the family Hydrocharitaceae can be traced back to 1880, when Fischer observed the development of the embryo sac in *Helodea canadensis* and reported the Polygonum type. Further attention on the morphology of the family was drawn by the interesting report of Kerner (1891) on pollination in *Vallisneria spiralis* L. and resulted in renewed discussions (Wylie, 1917; Wager, 1928; Svedelius, 1904 and 1932; Kausik, 1939). The older embryological information has already been summarized by Schnarf (1931). Subsequent to the publication of this treatise, the following studies have appeared:

Hydrilla verticillata (Maheshwari, 1933)
Vallisneria spiralis (Rangaswamy, 1934; Witmer, 1937; Maheshwari, 1943)
Ottelia alismoides (Narasimha Murthy, 1935; Maheshwari, 1943; Islam, 1950; Haccius, 1952)
Halophila ovata (Kausik and Rao, 1942)
Enalus acoroides (Kausik, 1940 and 1941)
Stratiotes aloides (Baude, 1956)
Blyxa echinosperma (Rangaswamy, 1941). *B. oryzetorum* (Govindappa and Naidu 1956)
Lagarosiphon alternifolius (Padhya and Miss Haripriya Rao, 1960)

The present paper incorporates the results of investigation on *Blyxa octandra.*

Materials and methods

Material was obtained from the plants growing in the paddy fields round about Madras and fixed in Craf solution. Serial micro-

tome sections were stained with Heidenhain's iron-alum haematoxylin and counterstained with erythrosin or light green.

Observations

Microsporogenesis and male gametophyte : —

The hypodermal, multicellular archesporium cuts off periclinally the outer primary parietal layer and the inner primary sporogenous tissue (Fig. 1). The former undergoes a similar division and the inner of this again divides in the same plane to differentiate the inner tapetal layer and a wall layer (Fig. 2). Thus, the wall of the anther is made up of the outer epidermis and two middle layers limited internally by the tapetal layer. No fibrous thickenings are developed in the cell layer corresponding to the endothecium.

The tapetal cells are uninucleate, with densely staining cytoplasm. At the time of quadruplication of the microspores the tapetal cells begin to lose contact with one another (Fig. 3). The walls break down and the protoplasts of the tapetal cells migrate into the thecal cavity. The tetrads of microspores are surrounded by the periplasmodium, in which the tapetal nuclei are found scattered (Fig. 6). Late in the development of the male gametophyte the periplasmodium is completely used up.

The innermost wall layer gets crushed up at the time of the meiotic division of the microspore mother cells. The outer wall layer and epidermis remain intact till the shedding stage of the pollen.

The microspore mother cell undergoes reduction divisions which are accomplished in a successive manner. Though the bilateral arrangement of the tetrads is the predominant one (Fig. 5), decussate arrangement (Fig. 4) is also observed. The microspores soon get separated from the tetrad configuration.

Each uninucleate microspore is spherical with thick outer exine which soon develops spiny outgrowths. The microspore nucleus which at first is in the centre, now moves towards the periphery. The cytoplasm becomes confined to the peripheral region of the cell owing to the enlargement of the central vacuole. As a result of the division of the microspore nucleus, two cells result, a small generative cell delimited by its own deeply staining cytoplasm and a large vegetative cell with vacuolate, less dense cytoplasm (Fig. 7). The mature pollen grain is two-celled.

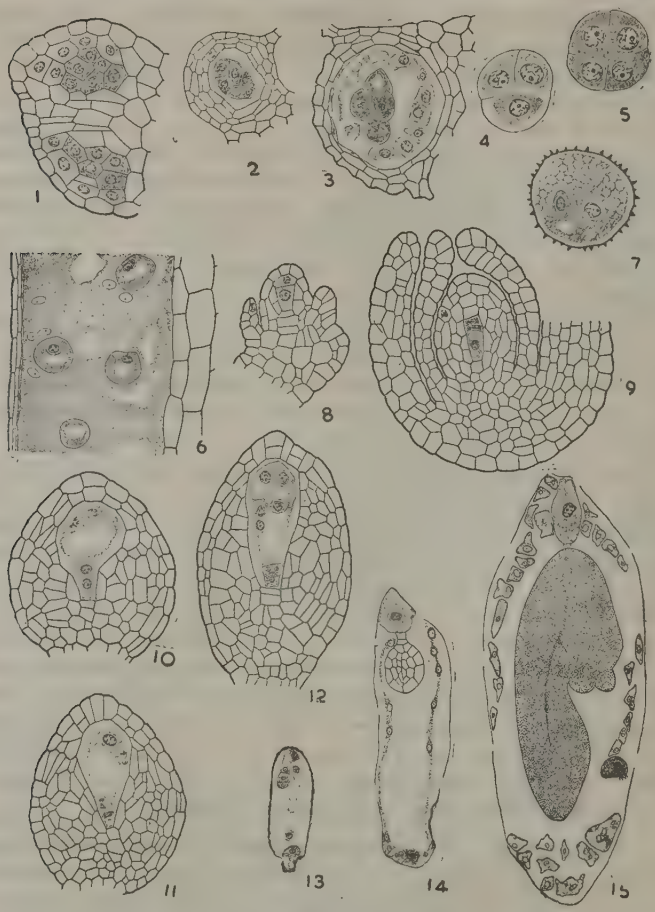
Megasporogenesis:—The inferior, tricarpellary, syncarpous, unilocular ovary consists of a large number of anatropous, bitegmic and crassinucellate ovules. The ovular primordium arises as a tiny protuberance from the inner wall of the ovary since there is nothing that may be recognized as placenta. The primordium is at first at right angles to the longitudinal axis of the ovary and soon enlarges and bends so as to attain the typical anatropous position. Even before the origin of the inner integument, the single hypodermal archesporial cell is differentiated. This cell divides by a periclinal wall to form primary parietal cell and a megaspore mother cell (Fig. 8).

The outer integument is initiated more or less simultaneously with the division of the primary parietal cell. This integument does not contribute to the formation of the micropyle till fertilization. It becomes a 3-4 layered structure whereas the inner integument is uniformly two cell layers in thickness.

The primary parietal cell generally divides vertically but periclinal division is also noticed occasionally. In addition to the parietal cell, the epidermal cell also divides, as a result of which the megaspore mother cell appears as if deeply seated in the nucellus (Fig. 9). The megaspore mother cell enlarges and divides to form the dyad cells of which the one towards the micropylar end becomes obliterated. The sister dyad cell enlarges and undergoes a periclinal division of which the upper derivative cell also meets with the same fate as that of the micropylar cell of the dyad. The tetrad stage is thus represented generally by "a row of three cells" (Fig. 9). At this stage two parietal cells placed one above the other and two similarly placed derivative cells of the epidermis can be observed anterior to the groups of megaspores.

The nucleus of the chalazally disposed megaspore undergoes divisions in the usual way to produce an eight nucleate embryo sac. The divisions of the nuclei at the opposite poles do not synchronize (Figs. 10, 11.). The mature embryo sac is soon organized, the two polar nuclei remaining nearer to the egg apparatus (Fig. 12); the antipodals are ephemeral.

Endosperm:—The pollen grains are not carried by the male flowers to the stigmas of the female flowers as in *Vallisneria* but they are shed and the free pollen grains as such float on water and reach the stigmatic surface. The course of the pollen tube is porogamous as has been described for other taxa of the family and normal fertilization is accomplished.



FIGS. 1-15.

1. Transection of an anther thecum, showing the primary parietal layer and the primary sporogenous tissue. x535. 2. Transection of an anther lobe depicting the two middle layers and the tapetum. x320. 3. Same; Microsporogenesis. x320. 4, 5. Tetrads of microspores. x775. 6. Part of the loculus in longisection showing the periplasmodial behaviour. x310. 7. Two-celled pollen grain. x775. 8. Longisection of the ovule; archesporial cell has divided to form primary parietal and megaspore mother cells. The outer integument is just originating. x320. 9. Linear tetrad of megaspores. x. 320. 10. Prior to the 4-nucleate stage of the embryo sac. x320. 11. Prior to the 8-nucleate stage of the embryo sac. x320. 12. Mature embryo sac. x320. 13-15. Longisections of the

The first division of the primary endosperm nucleus segments the embryo sac into a smaller chalazal chamber and a larger micropylar chamber. The nucleus of the micropylar chamber undergoes a series of free nuclear divisions. (Figs. 13, 14). Cell formation commences in the endosperm from the micropylar pole.

The chalozal corenocytic part of the endosperm pushes itself progressively towards the chalazal end destroying the neighbouring cells of the ovule and during this process grows past the haustorial basal apparatus. As a result, the latter cell appears to occupy an apparent lateral position (Figs. 16-18). It must be emphasized here that it is not the basal apparatus that is involved in any migration but its ultimate position is caused by the chalazal-wise growth of the endosperm. It may also be noted that the basal apparatus, even after being by-passed, still shows the remnants of the cell walls of the chalazal regions with which the apparatus was in contact earlier (Figs. 13, 14). The endosperm in the micropylar chamber ultimately becomes uniformly cellular.

Seed coat:—At the time of fertilization, the outer integument is generally made up of 4 or 5 and the inner integument of 2 cell layers (Fig. 19). The inner tangential wall of the inner integument alone persists as a membrane in the seed. All the cell layers of the outer integument are involved in the construction of the seed coat. The outer epidermal cells exhibit the presence of starch grains and the cell walls develop tenuous radial striations. The cells intervening between the outer and inner epidermis of the integument develop uneven thickenings of the primary wall. Another noteworthy feature of the seed coat pertains to the development of localized protuberances over the surface. The origin of such echinate structure is traceable even at a stage when the ovule is mature. A cell of the subepidermal layer divides by a periclinal wall. The derivative cells generally divide by anticlinal walls. This is followed by anticlinal divisions in the epidermal cells that confront the subepidermal group of cells (Figs. 20, 21). The cells thus derived — both from the epidermis and the hypodermis — bulge out from the surface of the seed coat in the form of protuberances (Fig. 22).

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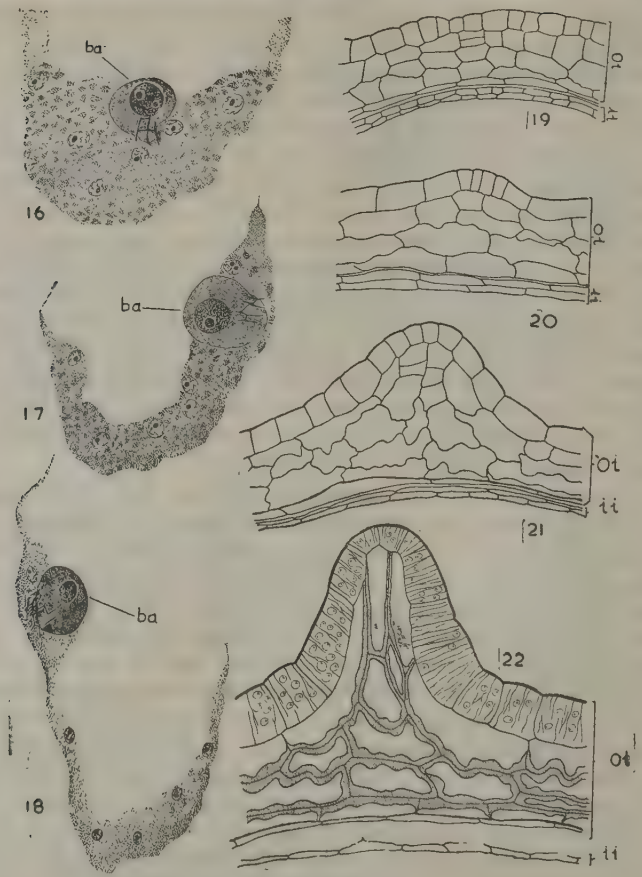
embryo sacs; illustrations of the stages in the development of the endosperm. x135. 13. Single celled chalazal endosperm chamber and two nucleate micropylar endosperm chamber. 14. Protoplasmic accumulation at the chalazal end of the micropylar endosperm chamber. 15. Cellular endosperm in the micropylar endosperm chamber. Apparent lateral position of the basal apparatus.



FIGS. 16-22.

16-18. Longisections of the chalazal portion of the embryo sac, showing the protoplasmic accumulation of the micropylar endosperm chamber and the apparent lateral position of the basal apparatus. x320. ba-basal apparatus. 19. Longisection of the integument at the time of fertilization. x310. 20-22. As above showing the formation of the protuberances. x310. ii-inner integument; oi-outer integument.

Discussion

Stamen:—The family Hydrocharitaceae exhibits rather a stereotyped embryological features. Still we can observe certain interesting points of differences in reference to some details. For

example, the development of the stamen is not uniform in all the taxa. In *Vallisneria* (Burr, 1903; Witmer, 1937) the single stamen arises from one primordium, splits up during ontogeny and results in two distinct stamens. A similar feature has been observed in *Nechamandra* (Lakshmanan, 1960). In *Blyxa,* however, each stamen arises from a single primordium and the structure matures directly without other morphological modifications. The development of the anther wall follows the course as described by Rangaswamy (1941) for *B. echinosperma,* but in *B.octandra,* the middle layers are two in number.

The microspore mother cells formed from the sporogenous tissue undergo meiotic divisions, during which time the tapetal cells lose their contact from one another to become organized into periplasmodium. This is a common feature of the family, the only exception recorded being that of *Lagarosiphon roxburghii* Benth. (Padhya and Miss Haripriya Rao, 1960). This observation, however, needs confirmation.

The tetrads of microspores are predominantly bi-lateral in arrangement. As in *Ottelia* (Narasimha Murthy, 1935) decussate arrangement is also often seen. The mature pollen grains show two-celled condition as in *Ottelia* (Narasimha Murthy, 1935), although three-celled shedding stage is the common feature in the family.

Ovule : — The derivatives of the single, hypodermal archesporial cell forms the parietal tissue and also the megaspore mother cell as in *Blyxa oryzetorum* (Govindappa and Naidu, 1956) whereas a parietal cell is reported to be lacking in *B. echinosperma* (Rangaswamy, 1941) and *Ottelia alismoides* (Narasimha Murthy, 1935). Islam (1950) has shown, however, that Narasimha Murthy's observation is erroneous. Baude (1956) following Narasimha Murthy's account on *Ottelia* states that the parietal cell is absent in *Stratiotes aloides.* Obviously, this author is unaware of the reinvestigations by Islam. The presence of a parietal cell thus appears to be a universal feature in the family. Generally there is only "a row of three cells" representing the tetrad stage. This is due to the immediate degeneration of the micropylar dyad cell. Some of the figures of Rangaswamy (Figs. 14-17, 1941) relating to the megaspores are misleading. They raise the doubt whether the "parietal cell" in his figure 14 is not formed by the anticlinal division of the epidermis, and the uppermost two megaspores in his figures 15-17 are not in reality the parietal cells.

Endosperm : — All members of the family exibit a helobial type of endosperm and *B. octandra* is no exception. The position of the primary endosperm nucleus varies with the taxa. It may be near the egg apparatus as in *Enalus* (Kausik, 1940), *Ottelia* (Narasimha Murthy, 1935) or in the centre as in *Vallisneria* (Witmer, 1937). Its position near the antipodals has been observed only in species of *Blyxa* (Govindappa and Naidu, 1956). My own observations indicate that the primary endosperm nucleus remains very close to the egg apparatus soon after triple fusion; after this is over, the nucleus moves towards the chalazal end. Such is the situation in *Ottelia* and *Vallisneria* also, which I have checked up.

The division of the primary endosperm nucleus which is followed by wall formation, results in a small chalazal endosperm chamber (basal apparatus) and a large micropylar chamber. There is no division in the former; it undergoes hypertrophy, probably functioning as a haustorial cell. The nucleus of the micropylar chamber undergoes a series of free divisions. At the time when the embryo has produced the first foliar appendage, wall formation begins in the micropylar part.

Another noteworthy feature of the endosperm in *Blyxa octandra* is the position of the basal apparatus (the chalazal endosperm chamber). In older stages it looks as if it occupies a lateral position in the sac due to the overgrowth of the chalazal part of the micropylar endosperm chamber deep into the chalazal nucellar tissue. The chalazal part of the endosperm also becomes cellular by now, although the tissue as a whole is very scanty. No remains of endosperm is seen in the mature seed.

Rangaswamy (1941) states that there is no cell wall formation in the micropylar endosperm chamber. This obviously appears to be an error.

Seed coat : — It is worthwhile to pursue the seed coat structure of the Hydrocharitaceae, as it is likely to yield significant data which may be useful in the classification of the genera within the family as will be shown in a subsequent contribution.

The seed coat in all the genera of the family is essentially constituted of the outer integument. The inner integument gets crushed and only the inner wall remains in *Vallisneria*, *Ottelia* and *Blyxa octandra*. But the surface of the seed is even in *Vallisneria*, with hairy outgrowths in *Ottelia*, and with undulations or spinescent

out growths in *Blyxa* and *Enalus*. In the genus *Blyra* the so-called echinate nature of the seed appears to be a generic feature. The seed coat of *Enalus* can be distinguished from the other taxa of the family by the persistence of the whole of the inner integument without any modification and formation of short, irregular, lobe-like indentations at the chalazal end.

Summary

Results of investigations on the gametophytes, endosperm and seed of *Blyxa octandra* are presented. In the microsporangium the primary parietal layer gives rise to two middle layers, and the tapetum; endothecium is not morphologically differentiated. Microspores are formed by successive division of the microspore mother cells. The uninucleate tapetal cells form the tapetal periplasmodium. The pollen grains are two-celled at the shedding stage.

The ovule is bitegmic. The archesporial cell by division gives rise to a parietal cell and a megaspore mother cell. The megaspore tetrads are generally represented by "a row of three cells". The chalazal megaspore develops into an eight-nucleate female gametophyte. Endosperm is of the helobial type. The wall formation in the micropylar chamber begins at the micropylar pole and extends towards the chalazal region. The chalazal coenocytic part pushes itself progressively, and during this process grows past the haustorial basal apparatus. As a result, the latter comes to occupy an apparently lateral position. The chalazal endosperm chamber (basal apparatus) enlarges and remain as a single cell.

The inner tangential wall of the inner integument persists as a membrane in the seed. The outer integument contributes towards the seed coat. The origin and development of echinate protuberances on the seed coat are described.

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A Redescription of *Lithidiocotyle secunda* Tripathi Monogenea and its Bionomics*

BY

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ABSTRACT

A redescription of *Lithidiocotyle secunda* Tripathi (1954) is presented. New combinations viz., *Lithidiocotyle elagatis* (Meserve, 1938, *L. meservi* (Yamaguti, 1953) and *L. australiensis* (Sandars, 1947) nom. nov. are made for *Gotocotyla elagatis, Gotocotyla meservi* and *Pseudomicrocotyle elagatis* respectively.

A detailed description of the clamp structure of *L. secunda* is presented; the extent of variations and their frequency of occurrence in the extensive collection made, are described; this critical assessment has led to the description of the possible presence of "polymorphism" in this group. A description of the clamp structure of *Lithidiocotyle bivaginalis* Ramalingam (1961b) (In Press) is presented to bring out the extent of variation seen in the clamp structure of *L. secunda.*

Egg, post-oncomiracidial larga and post-larva of *L. secunda* with 3 clamps on each side of the haptor are described. The evolution of the morphological characters with the growth of *L. secunda* is presented. As the time factor in growth is not known, the addition of clamps is taken to mean the growth of the worm. Some functional correlation between male-female activity and growth of the haptor and addition of clamps in *L. secunda* is presented.

The occurrence of "intrinsic metamerism" in monogenea is suggested and some observations are presented on the parasite communities in *Scomberomorus guttatus.*

INTRODUCTION

In the course of the investigation undertaken at the Central Marine Fisheries on the fish trematodes, the author, during the period from January 1954 to January 1956, examined 212 seer-fish, *Scomberomorus guttatus* (Bl. Schn.) of the size ranging from

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8·2 cm. to 95·8 cm. These fishes were obtained from shore seine nets, gill nets and hook and line. This extensive search yielded a large collection of the monogenetic trematode *Lithidiocotyle secunda* Tripathi (1954). As the description of *L. secunda* is incomplete and since the present study is based on a very extensive collection, it is deemed fit to present a redescription of it here.

A critical examination of the numerous specimens of *L. secunda* revealed that about 10% of the specimens showed some variations quite distinct from the rest of the specimens and they were consistently seen. The remaining 90% of the specimens were either unmodified or combined in them some of the distinctly different features of the minor (10%) group in different degrees. The 10% of the specimens when compared with the unmodified *L. secunda,* in the absence of intermediate forms, could lead to the creation of a separate species. The description of *L. secunda* thus includes the characters of variation seen in the 10% of the specimens and they are presented in italics with the figure of the adult for comparison. In addition, figures and description of the clamps in the minor group as well as in *Lithidiocotyle bivaginalis* n.sp.[1] are presented separately along with the figures and description of the clamps seen in 90% of the specimens in an attempt to bring out the extent and type of variation seen and to illustrate the possible presence of polymorphism in this group.

The numerous specimens of *L. secunda* obtained include from the smallest individual having in all six clamps to the largest adult having in all 269 clamps. This collection facilitated a study of the development of morphological characters with growth of the worm. Since the time factor in the evolution of the morphological characters is not known in the present case, the addition of clamps is taken to represent growth of the worm. But the number of specimens belonging to the different clamp series is much limited, very often to one, and hence an account of the morphological characters seen at the earliest and latest stages are described with reference to the addition of clamps.[2] These observations on the evolution of morphological characters facilitated a further study of

1. *Lithidiocotyle bivaginalis* n.sp. is described by the author elsewhere and it differs from the other known species of this genus in the possession of two vaginae opening by two separate pores.

2. The number of clamps mentioned relates to the total number present in the haptor.

the gonadial activity and a possible correlation on the growth of the haptor and the addition of clamps.

Besides the genus *Lithidiocotyle* genera *Pricea* and *Thoracocotyle* and parasitic copepods of the genus *Caligus* and a lerneaopodid copepod were also obtained from the same host. As it was observed that these fishes harboured on their gills very often more than one kind of parasite, a brief mention is made here on the parasite communities that occur on this host.

LITHIDIOCOTYLE SECUNDA TRIPATHI, 1954

The worm is elongate with body dorsoventrally flattened; its maximum width is one-tenth of the total length either across the region of the ovary or across the anterior region of the testes from whence it tapers both in front and behind. Its width across the region of the vaginal opening is half the maximum width; and the sides of the body are almost parallel from the region in front of the vaginal opening upto the level of the pharynx. This region forms something like 'neck' to the body and its junction is at the level of the vaginal opening. Posteriorly at the hinder region of the testes the width is approximately three-fourths the maximum width. The worms have a total length from 2·41 mm. to *12·87 mm.* and the haptor is from two-fifths to *half the total length.* Width of the haptor at its proximal region is three-fourths the maximum width. It narrows gradually; its width at its middle region is half the maximum width. The haptor ends distally in a terminal lappet measuring from 112μ to 201μ bearing a pair of anchors whose length ranges from 36μ to 55μ. Clamps are of the gastrocotylid type and details of their structure are presented later. In normally extended forms, for example, measuring 2·41 mm. and 7·17 mm.. there is a total of 79 and 269 clamps respectively. Some are *symmetrical* while others are asymmetrical. This is the first record of the presence of symmetrical and asymmetrical clamps to be described in the same specimen. The clamps are arranged in a single file in the majority of the specimens; but considerable number of specimens are present in the collection in which the clamps are arranged in a double file on each side of the haptor extending for a short distance either at the distal end, middle or at the proximal region. In a few specimens clamp rows on either side appear double; the clamps, however, are not strictly paired in the two double file, for their bases are in a single file, though due to excessive overcrowding they may slightly zig-zag but alternate pedicels carry their clamps diagonally inward so that two clamps come

to lie in the horizontal line forming an effective double file on each side. In extreme cases of elongation the double row is reduced to a single series. In addition the clamps show morphological variations. Though normal clamps are present in the majority of specimens, considerable percentage of the specimens have transversely elongated clamps as well as asymmetrical with much more heavy cuticularization of the type seen in *L. bivaginalis* in varying numbers and their position on the haptor is also very varying. These aspects are dealt with later.

Mouth is subterminal and ventral. Buccal suckers are oval and aseptate. Their size range from 24 × 39μ to 30 × 61μ. The size of the pharynx ranges from 39 × 24 μ to 64 × 39 μ. Oesophagus bifurcates near the region of the vaginal sucker, situated at a distance of one sixth of the total length from the anterior end. The intestinal crura extend into the haptor to a maximum of about one-fifth of its length and terminate blindly.

Gonads occupy either *the posterior third of the body and it is in front of the haptor* or occupy the second quarter of the worm from the anterior end and extend half way into the third quarter also. Testes number ranges either from 43 to 112 or *142 to* 298 and their size range either from 45 × 28μ to 130 × 81μ or *15 × 15μ to 95 × 52μ* or 45 × 28μ to 130 × 81μ. They extend to about one-fourth of the total length in both cases. They occupy the posterior half of the second quarter and extend into the anterior half of the third quarter of the worm. Hinder one-sixth of the testicular region is overlapped by the proximal region of the haptor. The cirrus is armed with numerous spines 17μ to 21μ long and their distal ends are pectinate. In the partly everted condition the cirrus is from 108μ to 196μ. The cirrus sac is either from *0·20 mm. to 0·52 mm.* or from 0·206 mm. to 0·345 mm. long. It is situated about half the distance from the anterior end to the vaginal opening. Two pairs of deeply stained bodies are present on either side of the cirrus sac at the base of the cirrus. Male pore is median and ventral. It is situated at a point one third the length from the anterior extremity to the intestinal bifurcation.

The size of the ovary is either from 30 × 33μ to 108 × 102μ or *45 × 116μ to 62 × 170μ*. The extent of the oviducal field ranges from half to the entire length of the testicular region. In exceptional cases as seen in 10% of the forms the oviduct extends as far behind the vaginal sucker and the length is greater than the testicular zone by a fifth. The ascending limb has 4 to 12 coils and the

descending limb has 8 to 16 coils. The distal end of the oviduct is directed backwards. It is continued by a narrow duct which is enlarged into the ootype. It is surrounded by shell glands. Beyond the ootype the oviduct is continued as the uterus which is median and opens on the ventral side just posterior to the male pore. A maximum of two eggs were found at a time. The egg shell of one is 145μ long with anterior and posterior filaments 294μ and 309μ respectively while another egg has the egg shell 197μ long with anterior and posterior filaments 257 μ and 207 μ respectively.

Vitelline follicles extend as two lateral bands on the outer sides of the intestinal crura from about the middle level of the vaginal sucker and their width narrows as they extend posteriorly and behind the mid testicular region they become sparser and a few scattered follicles extend into the proximal region of the haptor. The transverse vitelline ducts either *originate at the same level about one-fourth the distance from the oviducal field and join to form the common vitelline duct about half the distance from the beginning of the oviducal field* or lateral vitelline ducts originate at different levels; the right originates approximately at the level equal to two-thirds the length of the oviducal field and the left half way down from the level of the right duct to the posterior extremity of the oviducal field. They join to form the common vitelline duct about one eighth of the oviducal length from the posterior extremity of the oviducal field. The common vitelline duct opens at the ootype. Vaginal opening is median and dorsal. It is surrounded by well developed muscles and its size ranges from 108 × 174μ to 135 × 215μ. It is situated at the junction of the neck with the body about one-sixth to one-fifth of the total length from the anterior end. It leads to a narrow canal which runs behind close to the inner side of the left caecum and joins the oviduct just posterior to the ootype. At the level of the distal end of the oviduct it is enlarged into receptaculum seminis and its size ranges from 35 × 18 μ to 43 × 24 μ. Genito-intestinal canal is prominent, extends obliquely in front of the ovary; it opens at the ootype at one end and at the other end at the right caecum as in other Polyopisthocotylea.

While erecting the genus *Pseudomicrocotyle* Sandars (1947) compared her genotype with the species of *Microcotyle* van Beneden & Hesse (1863) but made no reference to *Lithidiocotyle* Sproston (1946). No detailed study of the clamp was made by her either. However she figured the asymmetrical struts forming the "V"

FIGS. 1-10.

FIGS. 1-10: *Lithidiocotyle secunda* Tripathi, 1954.

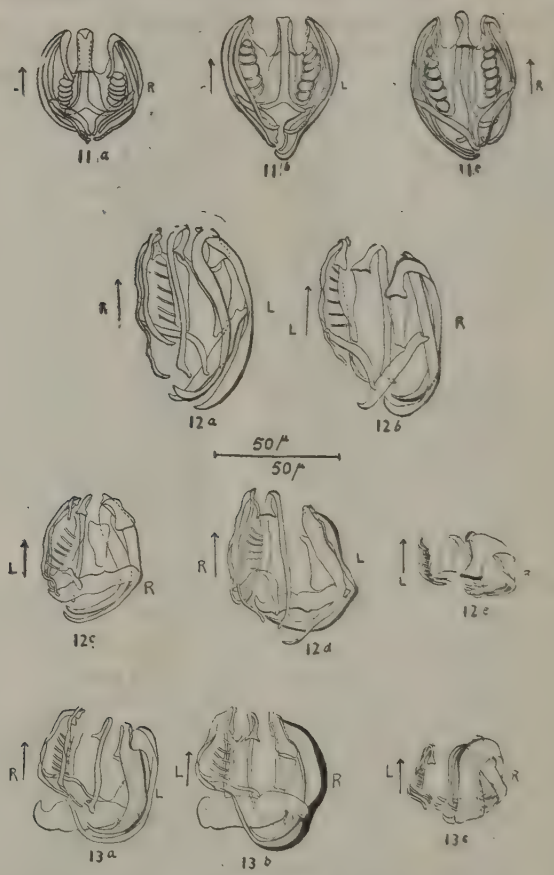
1. Entire worm from the 90% of the specimens (ventral view). 2. Enlarged view for the gonadial region and their ducts (ventral view). 3. Cirrus and cirrus sac. 4. Anchors. 5. Clamp anlagen arranged in a single file in the region proximal to the incipient clamps in *L. secunda* from the 90% of the specimens where in the clamp row is also in a single file. 6. Clamp anlagen arranged in a double file in the region proximal to the incipient clamps in *L. secunda* from the 90% of the specimens wherein the proximal region shows a double file. 7. *Lithidiocotyle secunda* Entire worm from the 10% of the specimens (dorsal view). 8. Egg of *L. secunda*. 9. Post-oncomiracidial larva of *L. secunda*. 10. Post-larva of *L. secunda* with 3 clamps on each side.

shaped struts characteristics of gastrocotylid clamps. A comparison of the clamp structure of *Lithidiocotyle acanthophallus,* (MacCallum & MacCallum, 1913), *L. secunda* Tripathi (1954) and *Pseudomicrocotyle elagatis* Sandars (1947) shows that all the three have similarly complex modification of the typical clamps of the family Gastrocotylidae to which *Pseudomicrocotyle* must be transferred. In addition *Pseudomicrocotyle* has the following characters such as the armed cirrus and median dorsal vaginal opening surrounded by well developed muscles shared by *Lithidiocotyle also.* Hence the genus *Pseudomicrocotyle* is to be treated as a synonym of *Lithidiocotyle* thus agreeing with Tripathi (1957) and its new designation is *L. elagatis* (Sandars, 1947) n. comb.

The clamps of *Gotocotyla meservi* Yamaguti (1953) also have two struts meeting in the form of a wide "V" in the posterodorsal part of the clamp capsule which includes it in Gastrocotylidae. But the clamps all show the kind of asymmetry presented by *Lithidiocotyle* and also in all the details of the clamp structure it is in agreement with *Lithidiocotyle;* hence it must be designated as *L. meservi* (Yamaguti, 1953) n. comb.

Sproston (1946), while dealing with *Gotocotyla acanthocybii* Meserve (1938) and *G. elagatis* Meserve (1938), had indicated that the clamp skeleton in these two species required further investigation and that it would probably lead to their removal to another genus. Yamaguti (1953), while creating a new species of *Gotocotyla* for the specimens obtained from the gills of *Elagatis* sp., had compared it with *G. sawara* Ishii (1936) and Meserve's species but did not compare it with either *Pseudomicrocotyle* Sandars or *Lithidiocotyle* Sproston. From the study it is clear that the clamp skeleton indicates that the species from *Elagatis* are congeneric with *Lithidiocotyle* so that *Gotocotyla elagatis* Meserve (1938) is designated as *L. elagatis* (Meserve, 1938) n. comb. The law of priority makes *L. elagatis* of Meserve to be retained and a new name is to be given for *L. elagatis* of Sandars. The species name given relates to its country of origin and is designated as *L. australiensis* nom. nov.

Although the clamp skeleton of *G. acanthocybii* Meserve (1938) is of the gastrocotylid type, the clamps are apparently symmetrical which excludes it from *Lithidiocotyle* apart from the male terminalia (absence of spiny cirrus). Hence it is retained as such. The following list gives the new combination, their host, locality



FIGS. 11-13.

11a. Symmetrical clamp in the 10% of specimens belonging to *Lithidicotyle secunda* (Dorsal view). 11b. Asymmetrical clamp in the 10% of specimens belonging to *L. secunda* (Ventral view). 11c. The same (Dorsal view). 12a. Normal clamp in the 90% of the specimens belonging to *L. secunda* (Ventral view). 12b. The same (Dorsal view). 12c. L. bivaginalis-type clamp in the 90% of the specimens belonging to *L. secunda* (Dorsal view). 12d. The same (Ventral view). 12e. Transversely elongate clamp in the 90% of the specimes belonging to *L. secunda* (Dorsal view). 13a. Normal clamp of *Lithidiocotyle bivaginalis* (Ventral view). 13b. The same (Dorsal view). 13c. Transversely elongate clamps in *L. bivaginalis* (Dorsal view).

in which they were obtained and the original name with which they were described :

Present designation	Host	Locality	Originally known as
Lithidiocotyle elegans (Meserve, 1938) n.comb.	*Elagatis bipinnulatus*	Panama	*Gotocotyla elegans* Meserve, 1938.
L. australiensis nom. nov.	*Elagatis bipinnulatus*	Australia	*Pseudomicrocotyle elagatis* Sandars, 1947.
L. meserri (Yamaguti, 1953) comb.nov.	*Elagatis* sp.	Macassar, Celebes	*Gotocotyla meserri* Yamaguti, 1953.

Clamp Structure in two species of *Lithidiocotyle*

The clamp structure of *Lithidiocotyle* namely *L. secunda* and *L. bivaginalis* agrees in the fundamental gastrocotylid type but differs in details. A comparative account of the clamp structure of the two species is presented together in the following description. This is done to help in the understanding of the variation of clamp shape (and within limits of structure also) present in *L. secunda* as compared with the 10% of specimens of *L. secunda* as well as *L. bivaginalis.*

Ten percent of the specimens of *L. secunda* studied by me showed the following type of clamp skeleton. The clamps are all of the gastrocotylid type, but they are further modified in shape and in their development of extra sclerites. While some are symmetrical, others are asymmetrical. When asymmetrical, those on the left side are mirror images of those on the right side of the worm. This is the first record of the presence of symmetrical and asymmetrical clamps to be described (Figs. 11a, b) in a species.

The clamp within its capsule (like the cover of a book with the spine of binding anterior) naturally tends to gape open, due to the median spring, but is kept firmly closed by muscle, attached along the spring distally and passing through the peduncle to the haptoral surface proximally: the contraction of these clamp-spring muscles act in mechanical opposition to the elasticity of the median spring.

The various loops from which skeleton evolved (Sproston, 1945 *b*) may be likened, in the present type of clamp, to its ventral and dorsal jaws, the right and left moieties of which are slightly separated medially. The sclerites of the ventral jaws arch dorsally in the vestigeal dorsal loop, here (as in all microcotylid clamps),

represented by two inwardly projecting spurs: these form the shoulders under which the ends of the dorsal jaw articulate. The median spring usually has the dorsal arm less than half as long as the ventral, which extends to near the lip of the capsule and has a more deeply bifid end. The two oblique struts or 'braces' (which distinguish the gastrocotylid clamp from the simple microcotylid type) have a sliding articulation at their outer ends with the sclerites of the dorsal jaw. They stretch obliquely across the floor of the capsule and their median articulation varies, sometimes with the curved tips of the dorsal jaw, sometimes with the median spring, but as in *Lithidiocotyle* spp. and allied genera, they are free. In the closed clamp in profile view, the jaws can be seen projecting slightly as claws. In the highly modified clamps (Figs. 13a, b) they are bent and their tips, as are also the separate jaw-moieties, do form opposable hooks, the abaxial tending to embrace the adaxial moiety.

The relation of the accessory sclerites in the clamps of the 10% of specimens representing *L. secunda* and those in the remaining specimens of *L. secunda* and *L. bivaginalis* are as follows. It is now only *for the first time,* that the extensive collection of material from Indian scombroids has facilitated the complete elucidation of the comparative anatomy of these, the most complex attachment organs found on any of the invertebrates. The name of the genus was derived from the irregular pebble-like sclerites figured originally by MacCallum and MacCallum (1913) for his *Microcotyle acanthophallus* (see Fig. 111 in Sproston, 1946) and since then by Sandars (1947, Figs. 3 & 4), Yamaguti (1953, Pl. IX, Fig. 44), and by Hargis (1956, Pt. XII, Figs. 10, 14 & 19). In none of these figures is the relation of the parts clearly shown nor is it discussed by any of these authors in their text.

The plate-like sclerites in the body of the capsule do not arise *de novo* in this genus, but their simplest form is already developed in *Gastrocotyle indica* (see Subhapradha, 1951, Fig. 8a) where the dorsal arm of the median spring of the clamp is shown to bear two wide, but weakly cuticularized lobes extending freely forwards in the "floor" (= dorsal wall) of the capsule. This double extension can be likened to the hyoid apparatus between the "jaws" and sharing the same points of suspension. In (Figs. 11a-c) this double expansion of the dorsal end of the median spring can be seen to be articulated medially with its expanded end, whence it sweepes laterally as two splayed wing-like plates, supported ante-

riorly by the dorsal spur of the ventral jaw, and laterally for a distance, by the top of the sclerites of the ventral jaw. Thus, these splayed wings are tilted obliquely at the sides and form a lateral sliding wall to the capsule cavity when the "ventral jaw" is swung open. In the 10% of the specimens representing *L. secunda* these wings are perforated widely, and there are mere thickened bars separating the lacunae (windows, or Latin fenestrae). When the jaws are closed the wings are bent slightly upon themselves forming shallow scoops and the fenestrae become slightly crescentic (Figs. 11a-c), but when stretched to wide ellipses, the intervening bars may appear parallel (Fig. 12a). The fenestrae are more extensive laterally in the remaining 90% of the specimens representing *L. secunda* and always appear like a row of mere bars, but stresses in the asymmetrically modified clamp permit their development only on the adaxial side, and the wing-plate on the abaxial side loses its articulation with the dorsal end of the spring (Fig. 12b). In the 10% of the specimens belonging to *L. secunda* there are 6 to 8 fenestrae in the dorso-lateral wings and they are always present on both the abaxial and adaxial sides, in spite of the development of asymmetry in some of the clamps. Fully-formed clamps (those other than the most proximal few clamps) are longer than wide elongated heart-shaped or oval, and range from $32 \times 38\,\mu$ to $55 \times 61\mu$. The ventral and the middle jaws of the abaxial side of the clamp are relatively longer and broader than those on its adaxial side in the remaining 90% of the specimens representing *L. secunda* (Figs. 12a, b). Besides, the struts on its abaxial side are longer and broader than those on its adaxial side. Coupled with these the sclerites on the abaxial side of the clamp are much more heavily cuticularized thus rendering the clamp more asymmetrical than those present in 10% of the specimens representing *L. secunda*. The clamp size ranges from $55 \times 40\mu$ to $76 \times 55\mu$.

Clamp structure in *L. bivaginalis*: The ventral and the middle jaws on the abaxial side are more convex; their distal third suddenly narrows and forms a strong hook-like ending (Figs. 13a, b). The sigmoidal thickenings dependent on the median spring and the 'V'-disposed struts are present in all; but the dorsal wings present in the 10% of the specimens representing *L. secunda* are most strikingly modified in this species as seen also in the remaining 90% of the specimens representing *L. secunda*. Consequently, the wing-like plaque from the dorsal end of the spring as seen in the 10% of the specimens representing *L. secunda* becomes distorted and loses its articulation on the abaxial side

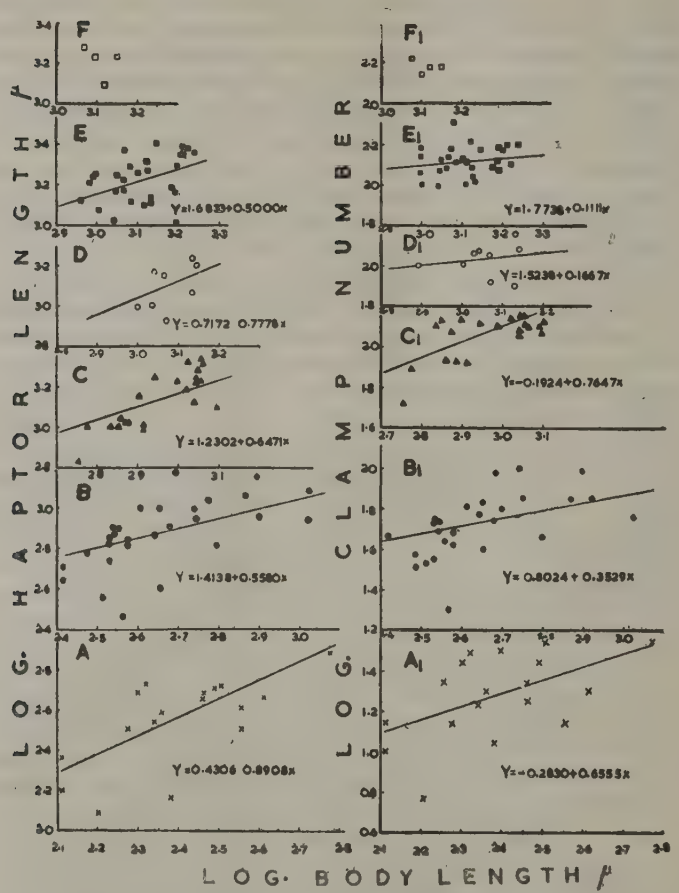


FIG. 14. Growth of the haptor and the addition of clamps in *Lithidiocotyle secunda* in the male phase.

Panel A represents the growth of the haptor in the post larva.
„ B represents the growth of the haptor in the immature.
„ C represents the growth of the haptor in the first male active phase.
„ D represents the growth of the haptor in the recovery phase after first male activity.
„ E represents the growth of the haptor in the second male active phase.
„ A_1 represents the addition of clamps in the post larva.

where the heavier musculature requires stronger support of the abaxial wing. In the remaining 90% of the specimens representing *L. secunda* and *L. bivaginalis* it has become a quadrilateral plaque articulating only with the tip of the dorsal arm of the ventral jaw on the abaxial side and the fenestrae have become scalariform bars on the adaxial side only. The articulation is obviously with the dorsal arm of the ventral jaw on adaxial side and sometimes it is also with the sigmoidal suspensor of the original wing from the adaxial side of the spring's dorsal end. The size of the clamp ranges from 39 × 36μ to 52 × 49μ.

The remarkable asymmetry of *L. bivaginalis*-type of clamps seen in development in the remaining 90% of the specimens representing *L. secunda* (Fig. 12 d) is a unique phenomenon. Their size is distinctly smaller (but more uniform in size 36 × 33μ to 45 × 42μ). Besides, the remaining 90% of the specimens representing *L. secunda* have transversely elongate clamps (Fig. 12 e) whose size ranges from 24 × 39μ to 33 × 58μ. Similarly *L. bivaginalis* has transversely elongate clamps (Fig. 13c) and their size range from 27 × 37 μ to 36 × 55 μ. The number of *L. bivanginalis*—type clamps, transversely elongate clamps and the type seen in the 10% of the specimens representing *L. secunda* present in the 90% of the specimens belonging to *L. secunda;* their position on the haptor, their frequencies either separately or in combination and the number of specimens in the above categories are dealt with in detail in the following chapter.

POLYMORPHISM IN MONOGENEA

While studying the life history of the monogenetic trematode, *Lithidiocotyle secunda* it was observed that the clamps showed remarkable morphological variations and that these were also very frequent in their occurrence. Hence, a detailed study of the structure of the clamps with particular emphasis on the range of morphological variability was made. The haptor besides possessing normal clamps (Figs. 12-a, b) that is, asymmetrical clamps

„ B_1 represents the addition of clamps in the immature.
„ C_1 represents the addition of clamps in the first male active phase.
„ D_1 represents the addition of clamps in the recovery phase after first male activity.
„ E_1 represents the addition of clamps in the second male active phase

which have moderate extra cuticularization), bears among them, two different types namely, (1) clamps accompanied by a maximum of secondary cuticularization *L. bivaginalis*-type clamps) (Fig. 12 d) and (2) transversely elongate asymmetrical clamps. (Fig. 12 e). Furthermore, variability was seen in the arrangement of the above clamps. All the same, the haptor does not show any variation in respect of its orientation to the body axis.

Thus it is observed from a study of 110 specimens belonging to *L. secunda,* that 86 specimens have their clamps arranged in a single file, and the remaining 24 have their clamps arranged in a double file on each side of the haptor.

Of the 86 specimens having the clamp arranged in a single file, 24 have clamps of a highly asymmetrical type (*L. bivaginalis*-type clamps), six have the transversely elongate asymmetrical clamps. In the 24 of the former group, the highly asymmetrical type of clamps are arranged either from the distal end as in 22 specimens; or from the middle region extending to the anterior most region, as in the remaining two specimens. Eighteen out of the 22 specimens have the highly asymmetrical clamps grouped together and are not found beyond the 30th clamp on the left side and not beyond the 25th clamp on the right side. In the remaining (four) specimens they are found to occur intermittently amongst normal clamps. In the latter group of six specimens, the transversely elongate asymmetrical clamps are found either near the distal end, as in two worms; or in the middle region, as in a single worm; or in the proximal region extending to the anteriormost region, in the remaining three worms. This type of clamps range from 2 to 29 on the left side, and from 1 to 20 on the right side. They are present from the distal end up to the 6th clamp on the left side and upto the 2nd clamp on the right side. In the middle region these are up to a maximum of 18 on the left side, and up to a maximum of 19 on the right side. In the proximal region they may extend to the anteriormost, formative region, with a maximum of 24 clamps on the left side and a maximum of 20 clamps on the right side. In some specimens this type of clamp is present only one side: on the right in three worms on the left in two worms.

The remaining 56 worms have normal clamps arranged in a single file thought to be characteristic of the 90% of the specimens representing *L. secunda.* It is very striking that

nearly 60% of the forms show various gradations in the assemblage of the morphologically varied clamps, as well as in the distribution of these on the haptor. Thus it is seen that the haptor of one and the same specimen shows marked, simultaneous variations both in kind, in relative position in a row and in the number of rows of clamps on one side of the haptor. In other words, the clamp exhibits individual polymorphism, while in the haptors are polymorphic in respect of the pattern of clamp arrangement.

Parallel examples of polymorphism can be drawn from the plant and animal kingdoms. In the butterfly *Colias*, the females are dimorphic, with either yellow or whitish wings. So also *Argynnis* where the females have their wings golden brown or olive green. Another example showing polymorphism is that of the land snail *Capaea* in which it is brought about in the course of evolution as a response to the visual perception of the predators. Yet another commonest type of polymorphism, is a seasonal one in *Daphnia* and its allies, as is also seen in the dinoflagellate *Ceratium tripos*, where the seasonal polymorphic forms were for a long time taken to be distinct species until the graded series were assembled.

The present case is quite different from the above examples, as well as from other known cases of polymorphism, in that polymorphism exhibited in *L. secunda* is both quantitative as well as qualitative, the latter to a limited extent. It is a case of polymorphism which is arhythmic or spasmodic unlike in the case of Cladocera which is rhythmic.

It is known that polymorphism has great biological significance since it proves selective differences between neutral characters. It also provides an original basis for adaptation to a wider range of environmental conditions than would otherwise be possible. Thus, the occurrence of the diverse forms among the normal ones plays an essential part in providing material for the selective effect of the complex factors in the micro-environment, and the evolutionary future is determined by this selective influence.

A collection of 156 specimens of monogenetic trematodes of the genus *Lithidiocotyle* taken from the gills of *Scomberomorus guttatus* has been intensively analysed. It is found that 90% of the specimens belong to *L. secunda* and the remaining 10%, though belong to *L. secunda* show some variations. In the 10% of the members the clamps are sessile and they are all arranged in double files on each side of the haptor. Both symmetrical and asymmetri-

cal clamps are present in one and the same specimen. The symmetrical clamps constituted only a very low percentage in all the worms. The asymmetrical clamps lack any secondary cuticularization, but show no loss in the typical cuticular elements of the clamps. In all the specimens studied, even the incipient clamps in the formative region are in a double file. The double-file arrangement of the clamps suggests a better and effective mode of securing attachment in the absence of peduncles.

In the 90% of the specimens of *L. secunda*, 72% have the clamps arranged in a single file on each side, but the remaining 28% of them have the clamps arranged in double files. The clamps in all the specimens have short peduncles, they are all asymmetrical and have suffered a loss of typical clamp elements on their abaxial side. Correlated with the pedunculated nature of the clamps, perhaps more "pull" is felt on the abaxial side of the clamp (of each side of the haptor), which is compensated by this secondary cuticularization. The absence of the double file arrangement of the clamps in the great majority of the specimens may indicate that the purpose served by the double file arrangement is now carried out by an advanced asymmetrical modification of a far stronger, single file of clamps. In the 72% of single-file specimens, the incipients in the formative region are also in a single file (Fig. 5). In those specimens having the clamps partly in a double file, in the proximal region, the incipient clamps in the formative region occur in a double file (Fig. 6) as seen in the case of 10% of the specimens.

During the entire period of this study there was one occasion only when fifteen adult specimens of *L. bivaginalis* were obtained from *Scomberomorus guttatus*. In these, the clamps are arranged in a single file on each side of the haptor. The clamps are pedunculated and are highly asymmetrical showing also an excessively abaxial cuticularization, and also of loss of some typical elements on the abaxial side of the clamps. Besides this type of clamps, transversely elongate clamps are present, but they were much fewer and only in a few specimens. The incipient clamps in the formative region are arranged in a single file in *L. bivaginalis*.

Thus it is seen that in the species of the genus *Lithidiocotyle* the clamps have not, apparently, reached any perfection, although highly asymmetrical clamps are more consistently seen in *L. bivaginalis*. Advancing a step further it is seen that the above two species fall clearly in three groups with reference to kinds of clamps

and their several arrangements (refer earlier), and among them 90% of the specimens of *L. secunda* occupy an intermediate position.

The importance of polymorphism in this instance appears to be towards an experimentation; this view is strengthened when we consider the substitution of the double-file arrangement of the clamps by an individually more modified single-file. It would seem that the advanced, asymmetrical modification of a stronger single-file of clamps carry out the function of the double file of relatively weak clamps, as indicated by a high percentage (72%) of the specimen (of *L. secunda*) having single-files of asymmetrical clamps accompanied by moderate cuticularization. That the forms of this species are tending towards achieving a still higher degree of asymmetry is seen from the fact that 41 out of 90% of the specimens of *L. secunda* have also (in varying proportions) the highly asymmetrical clamps of the type met with in *L. bivaginalis*. But the clamps typical of the 10% of the specimens of *L. secunda* are never found in the 90% of the specimens of *L. secunda* and in *L. bivaginalis*. On the other hand, 90% of the specimens of *L. secunda* show a turn towards the most asymmetrical type, hinting that the intermediate forms side by side with substituting the double file, are aiming at clamps of the highly asymmetrical type. In other words, it tends to prove that the intermediate clamp-types are precursors of the highly asymmetrical type, and the clamps of the first group (found in the 10% of the specimens of *L. secunda*) seem to be near the parent stock from which the other groups have evolved. The present observations indicate only that the entire assemblage of the intermediate group is in an evolutionary flux. It is not clear whether the line of advancement in the direction of the highly asymmetrical clamps, coupled with maximum secondary cuticularization (as seen in *L. bivaginalis*), is the culmination of this development, or whether it will show further changes in the course of evolution.

LIFE HISTORY OF *LITHIDIOCOTYLE SECUNDA*

Egg: The egg shell proper of *L. secunda* measures from 145μ to 197μ in length; and the anterior and posterior filaments measure from 227μ to 303μ and 136μ to 331μ respectively (Fig. 8). The eggs while in the uterus did not show as much development as was noted in the case of *Kannaphallus univaginalis* Ramalingam (1961 c). The anterior most eggs have not developed beyond

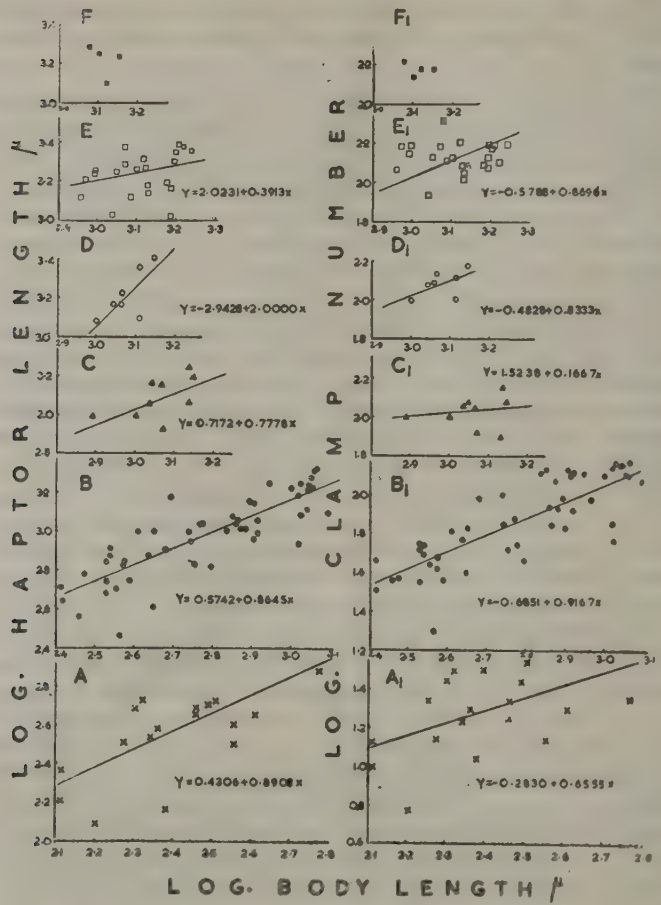


FIG. 15. Growth of the haptor and the addition of clamps in *Lithidiocotyle secunda* in the female phase.

Panel A represents the growth of the haptor in the post larva.
„ B represents the growth of the haptor in the immature.
„ C represents the growth of the haptor in the first female active phase.
„ D represents the growth of the haptor in the spent phase after first female activity.
„ E represents the growth of the haptor in the recovery phase after first female activity.

a few celled stage and those in the middle of the uterus are still in the undivided stage. Their size ranges from 20 × 18μ to 21 × 20μ. The eggs were isolated from the uterus of the worms and reared in the laboratory for a period of five days at room temperature (28°C—29°C) and by keeping them in the incubator at 33°C. They showed no signs of development and at the end of this period they all died. As only a few eggs were available in each specimen the experiment of artificially hatching them to the larval stage in the laboratory had to be abandoned.

The post-oncomiracidial larva: A single specimen of the post-oncomiracidial larva (Fig. 9) was obtained along with the juveniles and adults of *L. secunda*. It is likely that this larva might belong to that of *L. secunda*.

The post-oncomiracidial larva is 0·78 mm. long and its width is 0·24 mm. across the mid-oesophageal level; 0·15 mm. across the region of the body in front of the haptor. The haptor is not clearly marked off from the body as their lateral wings are folded inwards. The haptor still retains the gyrodactylid form, but the lateral marginal hooklets are lost. The haptor is 0·16 mm. long and 0·16 mm. broad. It possesses two pairs of anchors, the outer pair is 76μ long and the inner pair is 23μ long. In addition to these two pairs, a pair of hooklets are present in between the two dissimilar anchors on each side and they measure 12μ long.

The mouth is ventral. The size of the buccal sucker is 19 × 31μ. Though it is not known as to when the buccal suckers are formed due to the lack of larval stages earlier than the post-oncomiracidial larva, it is definitely clear that they are formed much earlier to the formation of first pair of clamps. But in *Diclidophora denticulata* the buccal suckers are formed by the time the first pair of clamps is formed (Frankland, 1955). The size of the pharynx is 45 × 30μ. The oesophagus is 0·16 mm. long and bifurcates into the two crura which extend to the posterior end of the body and extend into the haptor to one-eighth of its length, where they terminate

Panel A_1 represents the addition of clamps in the post larva.
„ B_1 represents the addition of clamps in the immature.
„ C_1 represents the addition of clamps in the first female active phase.
„ D_1 represents the addition of clamps in the spent phase after first female activity.
„ E_1 represents the addition of clamps in the recovery phase after first female activity.

blindly. Pigment granules are found to be deposited on either side of the intestinal crura whereas in *Dicilidophora denticulata* the pigments are seen at a much later stage, at the time when the first pair of clamps has formed (Frankland, 1955). Two pairs of glands are present on either side of the oesophagus, the ducts from them lead anteriorly on either side of the pharynx and open to the exterior through the head glands.

For the first time a smallest specimen of the genus *Lithidiocotyle* belonging to *L. secunda* was obtained and its description is as follows: —

It has three clamps on each side of the haptor (Fig. 10). Its length is 0·56 mm. and its maximum width is 76μ. The haptor is one-fourth the total length. The size of the distal clamp is 30 × 30μ. The terminal lappet is 37μ long; it bears three pairs of anchors, the larger outer pair is 39μ long and the smaller inner pair is 18μ long. The third pair is the two larval hooklets which are present in between the two dissimilar anchors on each side and its length is 12μ each.

Mouth is subterminal and ventral. The size of the buccal sucker is 23 × 15μ. Phrynx is oval and its size is 25 × 17μ. Intestinal bifurcation is situated at a distance approximately equal to one-fifth of the total length from the anterior end. The intestinal limbs terminate blindly at the junction of the haptor with the body.

The reproductive anlagen is seen as a single mass of deeply stained nucleated cells and its size is 30 × 28μ. It is situated immediately behind the anterior half of the worm. A narrow column of deeply stained cells representing the future course of the vas deferens and the uterus extends from the region in front of the anlagen to the region behind the intestinal bifurcation. Cirrus anlagen is not differentiated yet.

Clamp addition and growth: Since the time factor in the evolution of morphological characters is not known in the present case, the addition of the clamps is taken to represent the growth of the worm. As the number of specimens belonging to each clamp series is very much limited, very often to one, the account presented on the development of the morphological characters at the earliest and the latest stages mentioned with reference to the addition of the clamps.[3]

3. The number of clamps mentioned relates to the total number present in the haptor.

The genital anlagen is formed as a deeply stained mass of cells in the inter-crural field as early as in the worm having in all 6 and in exceptional cases this condition of the genital anlagen is seen as late as in a worm having 22 clamps. The differentiation of the genital anlagen into two portions (the anterior one representing the future ovary and the posterior representing the future testes) is seen as early as in worms having 17 clamps or as late as in worms having 36 clamps. The number of cells of the posterior mass representing the future testes ranges from 45 to 67 and their size ranges from $3 \times 3\mu$ to $6 \times 6\mu$. By a process of division they come to possess two to four cells, thus constituting the testicular primordia. This stage is reached as early as in the worm having 14 clamps or as late as 44 clamps. By further division these testicular primordia come to possess larger groups of cells in each testis. Such discrete testes are formed as early as in the worm having 20 clamps or as late as in the specimens having 102 clamps. Morulae are formed in the testes as early as in the worm having 48 clamps or as late as in the worms having 114 clamps. Testes become active (for the first time) by the time the worms acquire 53 clamps. In an extreme case a worm with 150 clamps is still showing the first male active phase only. But the majority of the worms have the testes active (for the first time) when they acquire 74 to 120 clamps. The spent phase of the testes and the subsequent development of the testes (after the first activity) are seen as early as in worms having 81 clamps or as late as in worms having 146 clamps but in the majority of the worms the clamp-range is 100—122. Testes thus become active (for the second time) when the worms come to possess a minimum of 86 clamps or a maximum of 206 clamps. But the majority of the worms have their testes active (for the second time) when they acquire 110 to 152 clamps. Since the number of specimens in the collection showing the spent phase and the subsequent recovery (after the second activity) are few, no general conclusions were drawn.

Testes: From a comparative study of the increase in the size of the testes, which directly affects increase in the length of the testicular region of the worm the following observations were made. The testicular region extends over a length ranging from 0·078 to 0·402 mm. by the time the testes become distinct. By the time they develop morulae, the length of the testes region extends further, ranging from 0·121 to 0·818 mm. When the testes are in the active phase (for the first time) the extent of the testicular region ranges from 0·429 to 1·408 mm. In the worms having the testes

spent and recovering, the testicular region extends from 0·711 to 1·446 mm. During the active phase of the testes (active for the second time) the length of the testes region ranges from 0·549 to 1·743 mm. It is observed that those specimens which are immature and have not become active for the first time the testes are anterior to the haptor and extend into the proximal region of the haptor in those specimens which have attained either maturity or are active for the first time. The second male active phase is easily recognized by the occurrence, in the uterus, of the malformed egg or by the remnants of vitelline materials.

Cirrus anlagen is not seen to be differentiated in the worm having upto 20 clamps. The future cirrus is indicated by the thickened mass of deeply stained cells measuring from 42μ to 80μ and it is seen as early as in the worm having 20 clamps or as late as in the worm having 46 clamps. Well developed cirrus armature measuring 64μ to 84μ with spines measuring 11μ to 13μ is found in the worm having 34 clamps and over, though exceptionally it is formed as early as in the 20 clamp-stage and measures 67μ long. The cirrus sac is seen as a thin sheath, surrounding the armature as early as in the worm having 20 clamps or as late as in the worm having 66 clamps. Well formed muscles are present in the wall of the cirrus sac by the time the worm has acquired 58 clamps.

Ovary : The size of the ovary is 23 × 91μ in the worm having 20 clamps and it is 55 × 88μ in the worm having 150 clamps. It appears that with the growth the size of the ovary does not seem to be affected much. However, the extent of the oviducal field increases considerably with growth. Thus the extent of the oviducal field measures 0·10 mm. in the worm having 20 clamps and it is 0·523 mm. long in the worm having 150 clamps. The oviducal field extends to a length of 0·442 to 0·694 mm. when the ovary is active for the first time and measures 0·51 to 0·56 mm.[4] when ovary is active for the second time.

The vaginal sucker is seen to develop in the specimens having 72 clamps and above. By the time the testes and the ovary are well developed, the genito-intestinal canal and the vitelline ducts are also formed.

4. As the specimens in the female active phase (for the second time) are represented by only four specimens the range for the oviducal field could not be a true one.

From a study of the gonads in *Kuhnia scombri, Plectancotyle gurnardi* and *Microcoyle donavini* Sproston (1945a & 1946) has reported that there is an alternation of sex phases. Similarly in *L. secunda* two male phases and two female phases are noted. It is also observed that *L. secunda* is protandric. The development of the vaginal sucker much later than the development of the cirrus and the vas deferens can be considered as an evidence to support the view. The final and the most convincing evidence of protandry comes from the data furnished below wherein it is pointed out that the number of immature specimens belonging to the same clamp-group showed wide difference in the male and female phases. The number of worms shared between the male and the female phases ought to be identical if they were to spurt into activity simultaneously as was indeed recorded by Frankland (1955) in *Diclidophora denticulata.*

It will appear from the account given earlier in this section that the growth phases are not strictly divisible into stanzas on the basis of the clamp addition. For example, the genital anlagen occurs in two masses in worms with clamps ranging from 17 to 36 in number and at the same time worms having 14 clamps and above (up to a maximum of 44—a clamp range which is below that of and is also partly represented by the earlier clamp range), show in them the next advanced stage of the gonadial development wherein the testicular primordia have begun to appear. Alvey (1936), Willey (1941) and Frankland (1955) have all recognized the overlapping of the immature and adult stages in terms of the length of the worm or on the basis of the time taken to reach the different stages. However, the use of clamp addition as a measure of the age of the worms facilitates an easier analysis by setting range for the different stages in Fig. 20 Ramalingam (1961 c).

MALE-FEMALE ACTIVITY AND GROWTH OF HAPTOR IN *L.SECUNDA*

Definitions of the post larva, immature, adult male and adult female are given by me Ramalingam, 1961 c. The presence of either malformed eggs or droplets of vitelline materials in the uterus indicate that the female phase was recently active. On the basis of comparative study of the immature and the adult specimens forming a continuous clamp series and thus representing the continuity in the animal growth; the estimation of the number of times

the gonads were active, with reference to one or the other sex phases, was made.

In a collection of 113 specimens the number of specimens representing the various stages of the male phase are as follows: 18 are post larvae; 28 are immature; 22 are male active (for the first time); 9 are male recovery (after the first activity); 29 are male active (for the second time) and 4 are male recovery after the second activity. Similarly the number of specimens representing the various stages of the female phase are as follows: 18 are post larvae; 53 are immature; 9 are active female (for the first time); 7 are spent female (after the first activity); 22 are female recovering (after the first activity) and 4 are active female (for the second time).

The correlation between the gonadial activity on the one hand and the growth of the haptor or the clamp number on the other hand, has been statistically analysed and is presented in figures 14 and 15. To minimise the error due to contraction the following procedure is adopted. Length of the body is multiplied with the average width of the body of the corresponding individual specimen and the square root of the multiplied product is taken to represent the length of the body. In this figure, the abcissae represent the log. body length of the worm in mm. and the ordinates represent log. length of haptor in mm. and the log. number of clamps in the male and female reproductive phases respectively. The data in this transformation were found to fit a straight line and on the basis of the equation for allometric growth $y = bx^k$ the formula for each curve is given.

Figures 14 and 15 represent growth of the haptor and the clamp addition respectively during the male and female reproductive phases. The formulae $Y = 0{\cdot}4306 + 0{\cdot}8908x$ (panel A); $Y = 1{\cdot}4138 + 0{\cdot}5580x$ (panel B); $Y = 1{\cdot}2302 + 0{\cdot}6471x$ (panel C); $Y = 0{\cdot}7172 + 0{\cdot}7778x$ (panel D); $Y = 1{\cdot}6833 + 0{\cdot}5000x$ (panel E) represent the growth of the haptor during the various stages of the male phase and the formulae $Y = 0{\cdot}2830 + 0{\cdot}6555x$ (panel A_1); $Y = 0{\cdot}8024 + 0{\cdot}3529x$ (panel B_1); $Y = -0{\cdot}1924 + 0{\cdot}7647x$ (panel C_1); $Y = 1{\cdot}5238 + 0{\cdot}1667x$ (panel D_1); $Y = 1{\cdot}7738 + 0{\cdot}1111x$ (panel E_1) represent the addition of the clamps during the same phase.

Similarly the formulae: $Y = 0{\cdot}4306 + 0{\cdot}8908x$ (panel A); $Y = 0{\cdot}5742 + 0{\cdot}8654x$ (panel B); $Y = 0{\cdot}7172 + 0{\cdot}7778x$ (panel C);

$Y=-2{\cdot}9428+2{\cdot}0000x$ (panel D); $Y=2{\cdot}0231+0{\cdot}3913x$ (panel E) represent the growth of the haptor and the formulae: $Y=-0{\cdot}2830+0{\cdot}6555x$ (panel A_1); $Y=-0{\cdot}6851+0{\cdot}9167x$ (panel B_1); $Y=1{\cdot}5238+0{\cdot}1667x$ (panel C_1); $Y=-0{\cdot}4828+0{\cdot}8333x$ (panel D_1); $Y=-0{\cdot}5788+0{\cdot}8696x$ (panel E_1) represent the addition of the clamps respectively in the various stages of the female phase.

With the worm reaching the first male active phase, a fall in the rate of growth of the haptor as well as in the rate of addition of the clamps when compared with the rates in the post larva is noticed. On the other hand there is an appreciable increase in the rates while the first male active phase lasts. There is a steady increase in the rate of growth of the haptor during the recovery period (after the first activity). At the time of the second active male phase there is fall in the rate obtained during the first male activity. But the rate of addition of the clamps however, is retarded at the same time. This trend in the rate of addition of clamps continues even into the second active male phase.

With the worm reaching the first female activity, the rate of growth of the haptor in the immature falls slightly in comparison with the rate in the post larva. There is, however, an increase in the rate of addition of the clamps on a similar comparison. But during the first active female phase, there is further a slight fall in the rate of growth of the haptor. In the rate of addition of clamps at this stage, however, there is a marked fall. During the spent phase of the female (after first activity) there is a spurt in the rate of growth of the haptor as well as in the of addition of clamps. During the recovery period when the rate of growth of the haptor shows a distinct fall, the rate of addition of clamps continues to show a rising trend. As the number of specimens in the second active female phase are only four, this study could not be extended any further.

The general impression conveyed by this analysis is that the rate of growth of the haptor and the rate of addition of clamps proceed in an allometric fashion. It also brings out at the same time the fact that there are periods of sudden spurts of active growth or of retardation which follow closely the several gonadial phases. The more conspicuous rise and fall in the rates of growth seen in the female phases is probably due to the difference in the duration of the male and female phases which follow each other.

A similar difference noticed in *Kannaphallus univaginalis* wherein the brevity of the male phase is indicated (Ramalingam 1961 c).

THE OCCURRENCE OF "INTRINSIC METAMERISM" IN MONOGENEA

Morphological and ontogenic evidences were brought out by me (Ramalingam, 1960) which reveal that the members of the superfamily Microcotyloidea have the potentiality of adding an unlimited number of clamps on the haptor and these are continued to be added with the growth of the worm. The clamps in the majority of the members are pedunculated. The movement of clamp sclerites is effected by a complexity of muscles—(1) intersclerite muscles attached to the clamp capsule (2) main-jaw depressors *via* the median spring (also serving as a ligament) (3) the main directive muscle of the capsule which is attached to the abaxial sector of the clamp capsule and is continued with the longitudinal muscle fibres from the spring capsule-hinge to haptoral-unit muscles, becomes confluent to form the two longitudinal trunks. The haptor, therefore, has two separately converging systems of longitudinal muscles which originate deep in the parenchyma in the gonadial fields.

The contraction of the peduncle as well as the movement of the clamp-sclerites is effected quite separately but in unison or only subdependently of the adjacent clamps. A rhythm of contraction can be watched passing successively up or down the clamp row; this might be effected either through the central nervous system, or in response to the stimulation provided by adjacent units locally *via* an autonomic complex. It is clearly metameric in every respect just as the parapodia of polychaetes or the legs of millipedes are. The repetition of the clamp-units in the axial succession (repetition of units rather than gross enlargement of one paired units) therefore can be considered as a case of *incipient metamerism* though the internal division of the body into complete segments has not been achieved. The occurrence of metameric segmentation in widely different groups such as Annelida and Chordata might have evolved independently and in a parallel way as a direct growth character.

The addition of the clamp-units in regular succession begins in the *larval haptor* and not in front of it (Ramalingam 1960) and in this respect differs from Annelida and Arthropoda,

in which new segments are formed anterior to the anal segment (Annelida) or immediately anterior to the pygidium (Arthropoda). As, and when, new units (clamps) are formed the first-formed pair is pushed backwards as in the Annelida and Arthropoda (where the anal segment of pygidium is pushed backwards as new segments are added in front of it).

PARASITIC COMMUNITIES IN *SCOMBEROMORUS GUTTATUS*

A total of 212 seer-fish *Scomberomorus guttatus* were examined during the course of the investigation and their size ranged from 8·2 cm. to 95·8 cm. *Lithidiocotyle secunda* predominated over members of the genera *Pricea* and *Throacocotyle* as well as the other species of *Lithidiocotyle* namely *L. bivaginalis*. The percentage of infection, on an average, is as high as 83% and they are infected by any one or all the three genera of parasites in varying proportions. The bulk of the remaining 17% of uninfected fish was constituted by fish of the smaller length classes (8·2 cm to 24·0 cm.). The maximum incidence of monogenean infection ever to be recorded in this study was 35 parasites in one fish measuring 51·3 cm. Among the three genera, *Lithidiocotyle* predominates in the infection, followed by *Pricea* and *Thoracocotyle* respectively, in the order of importance.

Genus *Lithidiocotyle*: In this genus *L. secunda* was the dominant species whereas *L. bivaginalis* was very rare which was very striking. From the gills of three fishes measuring 79·0 cm., 88·5 cm. and 89·2 cm. caught by hook and line and landed at Kundugal Pt. (Gulf of Mannar side) a total of eighteen specimens, including three juveniles, were obtained on only one occasion (on 24-1-1956). A similar observation was made by me (1961c) with regard to the genus *Kannaphallus* wherein the bivaginate forms of *K. virilis* Unnithan (1957) was recorded by me only once and a total of three specimens were obtained whereas numerous specimens of *K. univaginalis* Ramalingam (1961c) were obtained from the same host viz., *Caranx sexfasciatus*. The collection of the genus *Monaxine* from the gills of *Formio niger*, in my collection, though limited, showed a similar trend wherein the bivaginate forms of *Monaxine bivaginalis* Ramalingam (1961a) are fewer as compared with *M. formionis* Unnithan (1957).

Genera *Pricea* and *Thoracocotyle*: Species of the genera *Pricea* and *Thoracocotyle* were present almost in equal numbers on

fish whose length ranged from 8·2 cm. to 24·0 cm. But on fish whose length ranged from 25·0 cm. to 55·0 cm. *Pricea* spp. dominated over *Thoracocotyle ovale* Tripathi (1954) (the only species described). It was observed that the intensity of infection as well as the frequency of occurrence of the different parasites were higher in the fish caught in the shore seine nets compared with those caught by hook and line and gill nets. Wolfgang (1954) working on the digenetic trematodes of marine fishes made a similar observation.

In the course of an examination of a total of 71 fishes, measuring from 8·2 to 24·0 cm., it was observed that a total of 66% of the fish were infested with one or the other parasites of the above genera. Of the 71 examined, 34 were taken from the nets operated from the catamaran and landed at Thangachimadam on the Palk Bay side about 5 to 8 miles from the shore; 53% of this lot (34) was infected with three genera; 50% of which were *Lithidiocotyle,* 18% were *Pricea* and 10% were *Thoracocotyle.* The remaining 37 were taken from the shore seine nets landed at Kundugal Pt. of which 78% were found similarly infected; the percentage occurrence of three genera were 62%, 32% and 22% in the same order of the genera.

In the size range of the hosts examined (8·2 cm. to 24·0 cm.) all the genera of parasites were immature. In the fish of length ranging from 30 to 55 cm. caught in the shore seine nets and those caught in the gill nets landed at Thangachimadam had the above parasites in the mature condition. In one instance a fish measuring 95·8 cm. caught in hook and line, landed at Kundugal Pt. was parasitized by forty early and late post-oncomiracidial larvae of *Pricea* spp. besides six adult specimens of that genus. The larvae were in various stages of development. This suggests that the infection commences early in the life of the hosts.

Copepod parasites (*Caligus* sp.) were also found along with the monogenea on the gills of the entire range of fish from 8·2 cm. onwards up to 95·8 cm. In contrast to this, however, a lernaeopodid (Copepoda) was obtained from fish of a limited range of length (from 16·0 cm. to 43·0 cm.). It is not clearly understood how *Caligus* sp. and lernaeopodid could reveal in their occurrence such marked difference in the size range of the host they infect.

In a total of 28 fish of length ranging from 16·0 cm. to 24·0 cm. a total of 11 lernaeopodid copepods were collected from eight fish. In four cases they were present along with *Caligus* sp. The largest

number of lernaeopodid copepods obtained from a fish measuring 35·8 cm. was 12 and the largest number of *Caligus* sp. collected from a fish measuring 42·8 cm. was 15.

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It is with great pleasure, I tender my thanks to Dr. N. K. Panikkar, Fisheries Development Adviser, Government of India (formerly Chief Research Officer) for his kind help and guidance during the course of my work at the Central Marine Fisheries. I am indebted to Dr. S. Jones, for facilities kindly extended to me unreservedly during my work at the Station. I record my deep sense of gratitude to Miss Nora G. Sproston who had, during her brief stay in this department, given unselfishly her time and contributed towards the formulation of my ideas by thought provoking discussions based on her wide and familiar knowledge of this field. I am greateful to Shri K. Krishna Rao of the Statistics section of the Station for his help in the statistical analysis of the data. My grateful acknowledgement is due to the Ministry of Education, Government of India for the award of a Senior Research Scholarship.

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ABBREVIATIONS USED IN FIGURES

an.	anlagen	v.s.	receptaculum seminis
c.	cirrus	sh.g.	shell gland
c.s.	cirrus sac	t.	testes
g.	gland	ut.	uterus
g.i.c.	genito-intestinal canal	vg.	vagina
o.d.	oviduct	vg.s	vaginal sucker
ot.	ootype	vit.	vitellaria
ov.	ovary	vit.d.	vitelline duct

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On a new Species of the Genus *Lithidiocotyle* (Monogenea: Gastrocotylidae), its Juvenile and Immature Forms from the Gills of *Scomberomorus guttatus**

BY

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(Received for publication on 28-6-61)

ABSTRACT

Morphological description of *Lithidiocotyle bivaginalis* n.s.p. from the gills of *Scomberomorus guttatus* is presented and it differs from the known species in the presence of two vaginae, opening by separate pores surrounded by well developed suckers. Descriptions of the juvenile and immature *L. bivaginalis* are presented. A comparison of these stages with that of *Monaxine bivaginalis* Ramalingam (1961a) is presented. The rarity of the bivaginate forms of the genus *Lithidiocotyle* viz., *L. bivaginalis* was observed in the collection made over a two-year period from the gills of 212 *Scomberomorus guttatus* of the size ranging from 8·2 cm. to 95·8 cm.

In the course of the investigation on the trematode parasites of fishes undertaken at the Central Marine Fisheries, Mandapam, the author collected on one occasion from three *Scomberomorus guttatus,* measuring 79·0 cm., 88·5 cm. and 89·2 cm., eighteen monogenetic trematodes (including 3 juveniles) belonging to the genus *Lithidiocotyle* Sproston (1946). On a closer examination, they proved to be different from the known species of the genus by the possession of two vaginae, opening by two separate pores and hence they are here designated as new species *Lithidiocotyle bivaginalis* and their full description is presented. During the period from January 1954 to January 1956 a total of 212 *Scomberomorus guttatus* of the size ranging from 8·2 cm. to 95·8 cm., obtained from shore seine nets, gill-nets and hook and line were

* Formed part of thesis approved for the degree of Doctor of Philosophy of the University of Madras.

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examined and on no other occasion *L. bivaginalis* was recorded. But *L. secunda* Tripathi (1954) occurred in far more abundance and it was recorded either along with *L. bivaginalis* or *Thoracocotyle ovalis* Tripathi (1956) or *Pricea* spp. or in combination with the latter two species.

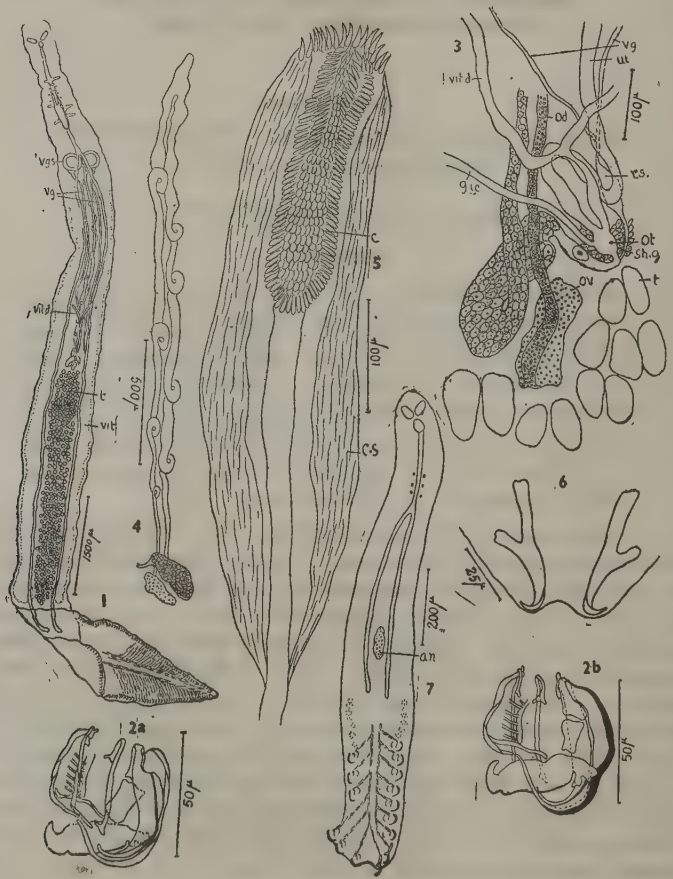

FIG. 1-7: *Lithidiocotyle bivaginalis* n.sp.

1. Entire worm (ventral view). 2a. Clamp (ventral view). 2b. Clamp (dorsal view). 3. Enlarged view of the gonadial region and their ducts (ventral view). 4. Ovary and the coiled oviduct. 5. Cirrus and cirrus sac. 6. Anchors. 7. Juvenile *Lithidiocotyle bivaginalis*.

Lithidiocotyle bivaginalis n.sp.

The worm is elongate with dorso-ventrally flattened body; its maximum width across the anterior region of the testes is up to one-eighth the total length. The sides of the body is approximately parallel from the level of the vaginal openings to the posterior region of the testes. The width of the body narrows in front of the vaginal openings and its width across the cirrus is half the maximum width. This region forms something like the 'neck' to the body and its junction is at the level of the vaginal openings. The worms have a total length of 4·15 mm. to 12·62 mm. The haptor is one-fourth the total length. It is triangular and leaf shaped. Its width at the proximal region is up to two times the maximum width of the body and half-way behind it is half that at the proximal region. The haptor ends in a terminal lappet, 52μ to 91μ bearing a pair of anchors whose length ranges from 52μ to 58μ. Slight asymmetry is noticed in the haptor, one side is longer with 61 to 80 clamps and the other side is with 52 to 74 clamps. In the twelve specimens studied six had the greater number of clamps on their left side. The clamps are of the gastrocotylid type; they exhibit maximum asymmetry and those on the left side are the mirror images of those on the right side of the worm. The ventral and the middle jaws on the abaxial side are more curved; their distal end suddenly narrows and forms a strong hook-like ending (Figs. 2a, 2b). The dorsal wings are strikingly modified as compared with the clamps of *L. secunda* (Ramalingam, 1961b). Consequently the wing like plaque from the dorsal end of the spring is distorted and loses its articulation on the abaxial side where the heavier musculature requires stronger support of the abaxial wing. It has become quadrilateral plaque only with the tip of the dorsal arm of the ventral jaw on the abaxial side and the fenestrae have become scalariform bars on the adaxial side only. The size of the clamps ranges from 39 × 36μ to 52 × 49μ.

Mouth is subterminal and ventral. Buccal suckers are asepatate and their size ranges from 142 × 46μ to 160 × 62μ. The size of the pharynx ranges from 85 × 96μ to 56 × 94μ. Oesophagus has diverticula on its lateral sides. Intestinal bifurcation is situated at a point equal to one-fifth of the total length from the anterior end. The intestinal caeca terminate either at the same level; ending just behind the last row of testes or ending at the proximal region of the haptor. Or they may terminate at different levels,

one limb ends at the level of the posterior row of testes and the other terminates in the proximal region of the haptor.

Gonads are situated anterior to the haptor and they occupy the second and third quarter of the worm from its anterior end. The number of testes ranges from 214 to 389 and they occupy the posterior two-thirds of the middle half of the body. Their size ranges from 48 × 46μ to 108 × 60μ. The cirrus is armed with numerous spines and the length of the contracted cirrus ranges from 0·173 mm. to 0·272 mm. The length of cirrus spines ranges from 18μ to 20μ. The length of the cirrus sac ranges from 0·228 mm. to 0·603 mm. and the thickness of the wall is from 35μ to 58μ. Three pairs of deeply stained bodies are present on either side of the cirrus sac at the base of the cirrus. The cirrus is situated about half the distance from the anterior end to the vaginal openings. Male pore is median and ventral. It is situated at a point half way from the anterior end to the intestinal bifurcation.

Ovary size ranges from 73 × 42μ to 176 × 70μ. The oviducal field extends to a length equal to half the testicular length. The ascending and descending limbs are coiled, each three or four times. The distal end of the oviduct is directed backwards and is continued by a narrow duct which enlarges into the ootype. It is surrounded by the shell gland. Beyond the ootype the oviduct is continued as the uterus which is median and opens just posterior to the male pore on the ventral side.

Vitelline follicles extend as two lateral bands on the outer sides of the intestinal crura from behind the region of the vaginal openings to the level of the posterior row of testes and their width is approximately the same throughout its length. The transverse vitelline ducts originate about half the distance from the beginning of the oviducal field. They unite to form the common vitelline duct three-fourths the distance from the beginning of the oviducal field. Two conspicuous circular vaginal openings, one on either side of the median line, are present on the dorsal side at the junction of the 'neck' with the body, about one-fifth of the total length from the anterior end. They are surrounded by well developed muscles and the size of the vaginal sucker ranges from 268 × 220μ to 323 × 270μ. The vaginal canals run close to the inner sides of the crura and the right canal traverses across, towards the left side about three-fourths of the distance of the oviducal field from the beginning of the oviducal field and joins the left canal. The common vaginal canal is continued backwards and opens at the oviduct

just posterior to the ootype. The common canal shows an enlargement serving as the receptaculum seminis and its size ranges from 78 × 25μ to 96 × 35μ. It is situated just in front of the distal end of the oviduct. The genito-intestinal canal is prominent; it extends obliquely in front of the ovary; and opens at the ootype. At its other end it opens at the right caecum.

Lithidiocotyle bivaginalis differs from *L. acanthophallus* (MacCallum & MacCallum, 1913) Sproston, 1946, *L. elagatis* (Meserve, 1938), *L. australiensis* (Sandars, 1947) Ramalingam (1961b). *L. meservi* (Yamaguti, 1953) and *L. secunda* Tripathi, 1954 by the possession of two vaginal openings and hence described as a new species.

Along with the adults, juvenile and immature *Lithidiocotyle bivaginalis* were also obtained. The former (Fig. 7) is represented by a single specimen having in all 13 clamps and the latter is represented by two specimens, one having in all 20 clamps and the other 36 clamps.

Juvenile *Lithidiocotyle bivaginalis* (Fig. 7) has a total length of 1·23 mm. with maximum width across the middle region of the body (0·19 mm.). Haptor is one-third the total length and has 13 clamps. The clamp size ranges from 18 × 18μ to 30 × 25μ. The terminal lappet has one pair of anchors, each 53μ long.

The size of the buccal sucker is 42 × 33μ. Pharynx size is 36 × 30μ. Intestinal bifurcation is situated at a distance equal to one-fourth of the total length from the anterior end. Intestinal caeca end in front of the haptor.

The reproductive anlagen is seen as a single mass of deeply stained cells and its size is 91 × 26μ and it is situated immediately behind the anterior half of the worm. Cirrus anlagen is not yet differentiated. Rudiments of vaginal suckers are not seen. Three pairs of deeply stained bodies are present at the region of the future cirrus.

Immature *Lithidiocotyle bivaginalis*

The two specimens with 20 and 36 clamps measure 1·33 mm. and 2·15 mm. respectively. The haptor is one third the total length. Terminal lappet bears one pair of anchors 45μ to 55μ.

Size of the buccal suckers ranges from 64 × 33μ to 76 × 42μ and that of the pharynx ranges from 39 × 24μ to 52 × 27μ.

Intestinal bifurcation is situated at a distance equal to about one-seventh the total length from the anterior end. Intestinal caeca terminate in front of the haptor.

Reproductive anlagen is seen in two groups: the anterior represents the future ovary and its size is 40 × 27μ and the posterior group represents the future testes and its size is 40 × 53μ. They extend to a length one-ninth of the total length of the worm and is situated in front of the haptor. A median column of deeply stained cells extends from the region anterior to the reproductive anlagen to the region posterior to the intestinal bifurcation. Cirrus anlagen is not yet differentiated. Three pairs of deeply stained bodies are present at the region of the future cirrus and they are present in the adult also. Rudiments of the vaginal suckers are not yet differentiated. A comparison of this with the immature *Monaxine bivaginalis* Ramalingam (1961a) described by me elsewhere (1960) reveals that in the latter the rudiments of the vaginal openings are seen much earlier in the development.

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ABBREVIATIONS USED IN FIGURES

an	anlagen	r.s.	receptaculum seminis
c.	cirrus	sh.g.	shell gland
c.s.	cirrus sac	ut.	uterus
g.i.c.	genito-intestinal canal	vg.	vagina
o.d.	oviduct	vg.s.	vaginal sucker
ot.	ootype	vit.	vitellaria
ov.	ovary	vit.d.	vitelline duct

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Studies on Indian Cryptonemiales—III*

Halymenia C. A. Ag.

BY

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(Received for publication on 30-4-61)

ABSTRACT

Full details of reproduction in the genus *Halymenia* are given for the first time. Of the four species of *Halymenia*, three species, *H. porphyroides*, *H. floresia* and *H. dilatata*, show close agreement in pre-and post-fertilization development.

In *Halymenia* spp., the carpogonial branches and auxiliary cells are formed in distinct accessory ampullae and are unicarpogonial. The carpogonial branch is two-celled. The carpogonial branch cell has a two to four-celled lateral sterile branch. The connecting filaments are formed by the fertilized carpogonium directly and each of them fuses with several auxiliary cells. Then the auxiliary cells cut off a cell, the gonimoblast initial, towards the top. By further activity a compact gonimoblast, which is made up of distinct gonimolobes, is formed but the limits of the gonimolobes become obscured in the later stages. The development of a nutritive basket is absent, but the entire ampullary cluster persists as a loose pericarp round the ripe cystocarps.

The fourth species, *Halymenia polydactyla*, differs from the rest and the details of development are in close agreement with that in *Sebdenia*. The carpogonial branches and auxiliary cells are remote and are formed from the intercalary cells of normal vegetative filaments and not from accessory ampullae. The carpogonial branches are three to four-celled and simple without a lateral branch. The connecting filament fuses with an auxiliary cell. The cells of the cortex surrounding the auxiliary cell develop into a special nutritive plexus following gonimoblast development. An arched wall is deve-

* In this third paper of the series the ontogeny of the cystocarp in the genus *Halymenia* is discussed. The genera *Grateloupia* and *Corynomorpha* have been dealt with in the first two papers of this series. These papers formed part of a thesis approved for the Degree of Doctor of Philosophy of the University of Madras.

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loped above the cystocarp by the activity of the outermost cortical cells above the auxiliary cell. The cystocarpic cavity is formed by the rupture of the cortex. In these characters the alga agrees with the Gigartinales to which it is transferred and placed under the genus *Sebdenia*.

INTRODUCTION

Berthold (1884) was the first to give an account of the reproductive structures in the genus *Halymenia*. In his monograph of the Cryptonemiaceae of the Gulf of Naples he has briefly described reproduction in *Halymenia floresia* (Clem.) Ag., the type species. He has also given a figure of an auxiliary ampulla seen from below, one of the fertilized carpogonium sending out connecting filaments, and two more figures showing the cutting off of the gonimoblast initial from the auxiliary cell after fertilization. Okamura (1908-1909) gives figures of an ampullary cluster in *H.acuminata* (Holmes) J.Ag. and a young gonimoblast in *H.formosa* Harv. (*H. durvillaei* Bory var. *formosa* (Harv.) W. van Bosse) without any discussion. Weber van Bosse (1921), in her list of Siboga algae, very briefly describes some features of reproduction in *H.durvillaei* and has also given a figure showing a young gonimoblast. In the same paper she also indicates that *H.dilatata* Zanard. resembles *H. durvillaei* in its reproduction. In his 'Marine red algae of Pacific Mexico', Dawson (1954a) describes briefly the auxiliary and carpogonial branch clusters as well as some details of development in *H.bifida* Dawson. He also gives figures illustrating the reproductive structures of this species and those of *H.abyssicola* Dawson. But details regarding post-fertilization development in the carpogonial ampulla are not known so far. Except in Dawson's (*loc. cit.*, p. 270) account, there is no mention of the organization of the auxiliary and carpogonial ampullae. In order to obtain some more information regarding these essential details, the writer took up for study the following four species occurring in India *viz.*, *H.porphyroides* Boergs., *H.floresia* (Clem.) Ag., *H.dilatata* Zanard., and *H.polydactyla* Boergs. The results of this investigation are set out in the following pages.

HALYMENIA PORPHYROIDES BOERGESEN

Material: Okha, 6th January, 1955, *leg.* T. V. Desikachary.

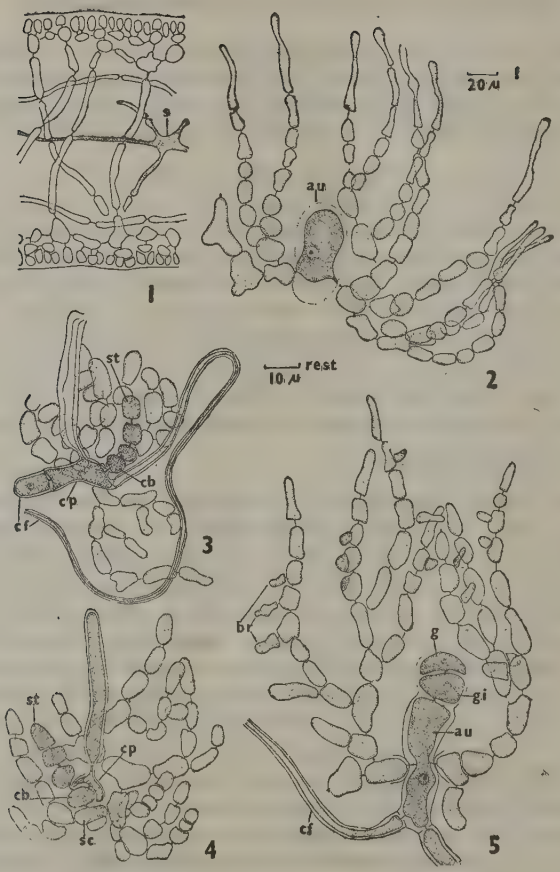
Halymenia porphyroides was described by Boergesen in 1932 based on specimens collected by him at Okha and Dwarka. The

material used for this investigation was collected at Okha, the type locality, and agreed well with Boergesen's specimens in the Iyengar herbarium at the Madras University. Both tetrasporic and cystocarpic specimens were present in the collection.

The alga grows attached to rocks and stones facing the open sea. Attachment to the substratum is by means of a small discoid holdfast from which the single erect foliaceous blade arises. The lamina has a short stipe, 5-10 mm long, which expands abruptly into the leaflike blade. The base of the lamina is broadly cordate and the blade itself is flat or undulate, broadly rounded, up to 48 cm. long and 41 cm. broad, simple, or at times more or less lobed (Plate VI Fig. 3). The margin is sinuate in without any proliferations. Plants are a fine rose red in colour and are tough and elastic in consistency, with a somewhat lubricous surface.

Structure of the thallus:

The thallus is multiaxial in construction, growth being of the fountain type. The blade is 230-300 μ thick. A section of the thallus shows a rather thin cortex and a large loose medulla; the medullary filaments are simple or branched, three to five cells long and run transversely or obliquely from cortex to cortex (Text-fig. 1). The width of the medullary filaments ranges from 10-12 μ. Interspersed with the medullary filaments are slender longitudinal rhizoids, 3-5 μ wide, and stellate cells 20-35 μ in diameter with arms up to 150 μ long (Text-fig. 1*s*). These stellate cells develop from the cells of the innermost cortical layer and are filled with a dense, deeply staining substance similar to what Norris (1957) has reported in the Kallymeniaceae. The cortex is three- to five-layered. The innermost layer consists of large irregular to substellate cells up to 15 μ in diameter and with arms of varying length. The medullary filaments are in direct connection with the cells of the innermost cortical layer. As mentioned already, some of them get enlarged and filled with a densely staining substance, later on getting also pushed into the medullary region. The middle layers of the cortex are composed of somewhat loose lying large and irregular cells, measuring 10-15 μ × 8-12 μ; at times the cells of the middle cortical layer are almost isodiametric. The outermost cortical layer consists of densely pigmented compactly arranged cells measuring 6-10 μ × 4-8 μ which form a continuous surface layer. Chromatophores are present in all cells of the cortex and in the medullary filaments, and are lacking only in the rhizoids and the large stellate cells. The chromatophores are somewhat long and



TEXT-FIGS. 1-5: *Halymenia porophyroides* Boergs.

1. Thallus in section showing internal organization. 2. Fully developed auxiliary ampulla; note that the auxiliary cell is an intercalary cell of the primary ampullary filament. 3. Fertilized carpogonium sending out two connecting fiiaments; note that the carpogonial branch cell (cb) and its lateral sterile branch (st) still preserve their identity. (The supporting cell is hidden below the carpogonial branch cell and the lowest cell of the lateral sterile branch). 4. Mature carpogonial ampulla; note the comparatively short and thick trichogyne. 5. Auxiliary ampulla showing an early stage in the development of the gonimoblast and the initiation of lateral branchlets (br) from the cells of the secondary ampullary branches.

(*au*: auxiliary cell; *br*: side branchlets; *cb*: carpogonial branch cell; *cf*: connecting filament; *cp*: carpogonium; *g*: gonimoblast; *gi*: gonimoblast initial; *s*: stellate cell; *sc*: supporting cell; *st*: lateral sterile branch).

ribbonlike in the cells of the medullary filaments and inner cortex; they get smaller in the middle cortical region and finally, in the surface layer, they are seen to be in the form of small irregular discoid structures.

Development of the carpogonial branches:

The carpogonial branches are developed in special ampullary clusters just as in the genus *Grateloupia*. These ampullary clusters arise from the middle cortical cells as lateral branches of secondary origin (Text-fig. 4). The primary ampullary filament is four- to five-celled, the cells being more or less cylindrical, 8-12 μ long and 3-6 μ broad. From the cells of the primary ampullary filament three- to five-celled erect secondary ampullary branches are developed; these secondary ampullary branches may be simple or forked. The cells of these branches also are cylindrical when young, but in fully developed ampullae, they are somewhat rounded, measuring 5-7 μ × 3-5 μ. The terminal cells are either cylindrical with the tip rounded, or slightly conical and up to 10 μ long and 4-6 μ wide. The fully developed carpogonial ampulla is 30-35 μ wide at the base and more or less globular but not flask-shaped as in *Grateloupia*.

The supporting cell of the carpogonial branch is an intercalary cell of the primary ampullary filament (Text-fig. 4 *sc.*). The carpogonial branch is two-celled, consisting of the carpogonium and a single carpogonial branch cell. Just as in *Grateloupia*, here the carpogonial branch cell has a short two- to four-celled lateral sterile branch (Text-fig. 4 *st*). The carpogonium is short conical, 7-10 μ wide at the base and 4-10 μ high. The trichogyne is rather short and stout as compared to that of *Grateloupia*. It is thick-walled, 4·5 -7 μ wide, and six to eight times as long as the carpogonium; a basal constriction is also noticeable (Text-fig. 4). The fully developed trichogyne pushes through the cortical cells and projects appreciably above the surface of the thallus. The primary ampullary filament is curved or coiled so that the carpogonial branch occupies approximately the centre of the ampulla.

Development of the auxiliary cells:

The auxiliary cells are also produced in ampullae which are accessory laterals of the middle cortical cells. The general constructional plan of the auxiliary cell ampulla is similar to that of the carpogonial ampulla (Text-fig. 2). But the auxilliary ampullae are much larger than the carpogonial ampullae and more

profusely branched. The primary ampullary filament is six to eight celled. The lower cells are somewhat irregular-subcylindrical, 5-8 μ wide and up to 20 μ long; the upper cells are almost subcylindrical, 5-6 μ wide and up to 10 μ long. The cells of the primary ampullary filament are never subspherical as in *Grateloupia*. Erect secondary ampullary branches arise from the cells of the primary ampullary filaments. These branches are six to ten cells long, usually simple, rarely forked once (or twice), and tapering to the tips. The cells of the secondary ampullary branches are subspherical when young, but in the fully developed ampullae they elongate and become cylindrical. The lower cells are 15-25 μ long and 6-8 μ wide and the upper cells are narrower, being only 3-3·5 μ wide and up to 10·5 μ long. The terminal cells of these branches are characteristically very long and narrow, 20-25 μ long and 2·5 μ broad, and have rounded or pointed tips (Text-fig. 2). The auxiliary cell is generally an intercalary cell of the primary ampullary filament. Even in young ampullae, it is easily distinguished by its larger size and denser contents. In the fully developed ampulla it is much larger than the rest of the cells. It is oblong with rounded ends, 20-30 μ × 12-15 μ, or rarely subspherical, 20-25 μ in diameter (Text-fig. 2 *au*). It is uninucleate, densely protoplasmic and has a thick wall. Fully developed auxiliary ampullae are about 50-60 μ wide at the base, narrowing to about 20-25 μ at the top and are 75-80 μ high. They are conical and flask shaped in marked contrast to the carpogonial ampullae, which are almost globular. On account of their large size, the auxiliary ampullae project into the medulla.

Post-fertilization development:

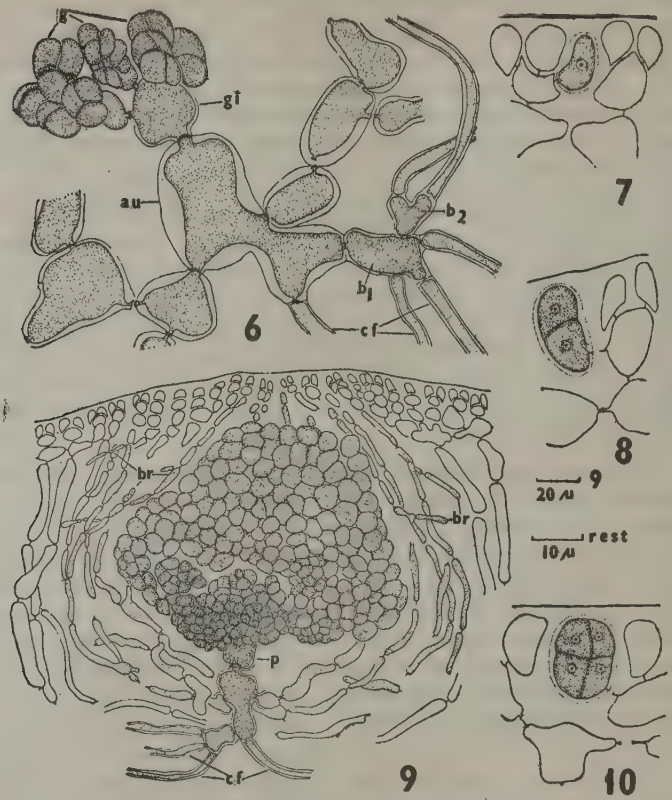
After fertilization, the trichogyne is first cut off. The carpogonium then enlarges to a greater or lesser extent and sends out a number of connecting filaments (Text-fig. 3). That it is only the fertilized carpogonium which has enlarged to form a densely protoplasmic irregular cell is seen very clearly particularly in cases where this enlargement is slight (Text-fig. 3). As is seen from text-fig. 3, the carpogonial branch cell (*cb*) and its lateral sterile branch (*st*) remain intact. In this respect *Halymenia porphyroides* resembles *Grateloupia*. The number of connecting filaments produced varies with the size of the enlarged carpogonium. Generally, the fertilized carpogonium enlarges considerably, becoming much lobed and irregular as in *Grateloupia indica* and in these cases up to ten connecting filaments may be produced. In some instances, however,

the enlargement is very slight and only two or three connecting filaments are sent out. The connecting filaments are thickwalled, non-septate and 3-4 μ in thickness. They run in all directions, seeking auxiliary ampullae and fusing with the auxiliary cells in them. After fusing with one auxiliary cell, the connecting filament almost always continues its growth and proceeds to other auxiliary ampullae and fuses with the auxiliary cells in them.

Here, as in *Grateloupia,* a gradual impoverishment of the cells of the carpogonial ampulla is noticeable during the enlargement of the fertilized carpogonium and the production of connecting filaments from it. This impoverishment also seems roughly proportionate to the size of the enlarged carpogonium, being less when there is only a slight enlargement and very great when the carpogonium is very much enlarged. This strongly suggests that the cells of the carpogonial ampulla play a nutritive role during post-fertilization development.

The connecting filament bulges at the point of contact with the auxiliary cell. The swelling is much more marked here than in *Grateloupia.* During later stages, this bulged out portion gets cut off by a cross wall from the rest of the connecting filament, though remaining in open communication with the auxiliary cell (Text-figs. 5, 6). It is from this bulged out portion that the new connecting filament grows out and continues its growth. Very often, a short cell is cut off at the base of the new continuation of the connecting filament. This cell soon swells, assuming a more or less rounded shape, and produces a number of additional connecting filaments which run out in all directions (Text-fig. 6 *b1*); occasionally, a second such cell may also be formed (Text-fig. 6 *b2*). It is interesting to observe that Berthold (1884, his Plate 8, Fig. 4), has shown such a cell at the base of the new connecting filament in *Halymenia floresia* from the Gulf of Naples, however, without making any comment. This is a feature not seen in *Grateloupia* with which there is close agreement in all other respects. It is obviously an attempt at greater dissemination of the effects of a single fertilization.

After fusion with the connecting filament, and presumably receiving a diploid nucleus, the auxiliary cell enlarges and cuts off the gonimoblast initial towards the surface of the thallus (Text-fig. 5 *gi*). The gonimoblast initial then produces a compact subglobose gonimoblast which is made up of distinct gonimolobes (Text-fig. 6 *g*). During later development, however, the consti-



TEXT-FIGS. 6-10: *Halymenia porphyroides* Boergs.

6. An early stage in the development of the gonimoblast, showing its division into three gonimolobes and also the continued growth of the connecting filament after its fusion with the auxiliary cell. Note the two cells at the base of the new connecting filament (b_1, b_2) from which secondary connecting filaments are issuing out. 7, 8 & 10. Stages in the development of the tetrasporangia. 9. Ripe cystocarp in section; note the compact gonimoblast, the prominent pedicel cell (p) and the pericarp formed by the persistent ampullary branches.

(*au*: auxiliary cell; *br*: side branchlets; *cf*: connecting filament; *g*: gonimolobes; *gi*: gonimoblast initial; *p*: pedicel cell).

tuent gonimolobes are not so clearly distinguishable as in *Grateloupia*. The lower derivative of the auxiliary cell continues to enlarge during gonimoblast development; the protoplasmic connections between it and the neighbouring cells become much wider. At times open communication is established between these cells and the lower derivative of the auxiliary cell, which is seen as a 'foot' (Text-fig. 9) at the base of the gonimoblast and forms, along with the pedicel cell (Text-fig. 9, *p*), a prominent feature of the cystocarp.

Side by side with gonimoblast development, the erect secondary branches of the auxiliary ampulla also produce very short lateral branches (Text-fig. 5 *br*), though not to the same extent as in *Grateloupia*. These lateral branches, together with the persistent secondary and primary ampullary branches form a loose envelope to the gonimoblast. They do not get used up during gonimoblast development as in *Grateloupia* spp., but persist as the cystocarp wall (Text-fig. 9 *br*).

The mature cystocarps are scattered, subspherical, and up to 200 μ in diameter, ostiolate and totally immersed in the thallus, jutting into the medullary region. The gonimoblast is very compact and subglobular, the gonimolobes being indistinct in the mature condition. But, the gonimolobes are clearly visible in the earlier stages of gonimoblast development (Text-fig. 6 *g*). The enlarged lower derivative of the auxiliary cell which still retains its original shape (the 'foot') and the pedicel cell are prominent features of the cystocarp; the persistent connecting filaments now appear like a cluster of rhizoids at the base of the gonimoblast (Text-fig. 9 *cf*). The ripe carpospores are irregularly rounded, about 15 μ in diameter, uninucleate and contain a number of short ribbon-shaped chromatophores.

Development of tetrasporangia:

Like the carpogonial and auxiliary ampullae, the tetrasporangia arise as accessory laterals from the cells of the middle cortex (Text-fig. 7). The tetrasporangium initial is cut off by a concave wall from the side or top of the mother cell. It soon enlarges and divides cruciately or decussately to form four tetrapores (Text-figs. 8, 10). Ripe tetrasporangia are rather small, oblong or ovoid, 12-15 μ long and 10-16 μ broad. The tetraspores are uninucleate, densely protoplasmic and contain several long ribbon-shaped chromatophores.

HALYMENIA FLORESIA (CLEMENT) C. A. AGARDH

Material: Tuticorin, growing on oyster shells, 21st December, 1952. *leg.* V. S. Sundaralingam.

Halymenia floresia is the type of the genus. It is almost world wide in distribution being known from the warmer parts of the Atlantic Ocean, the West Indies, Florida, the Mediterranean, the Canary Islands, and the Malayan Archipelago, etc. From the Indian region, it has been reported by Murray (1887) from Ceylon based on Fergusson's collections in the British Museum. Boergesen (1957a) has reported it from the Pearl Oyster beds, Tuticorin and the Chank Fisheries beds, Rameswaram, from specimens in the Iyengar collection sent to him (Plate VI, Fig. 4). The material used in the present investigation consisted of a single cystocarpic specimen collected from the Pearl Oyster beds at Tuticorin by Mr. V. S. Sundaralingam. This specimen agreed well with Iyengar's specimens in the Madras University Herbarium though it was much smaller in size.

The alga grows in deep water, attached to shells and small stones. Plants reach 30-60 cm. in height (the present specimen, however, was only about 25 cm. high), and are rose red in colour and soft and gelatinous in consistency. Attachment to the substratum is by means of a small disc-shaped holdfast from which the erect frond arises. The basal portion of the erect frond is almost stipitate, but this evenly expands into the leaflike blade which is very much divided, the ramification being tri- or quadripinnate, often irregular. The ultimate branchlets are long and narrow and gradually taper into the somewhat sharp pointed tips. The margin is entire and free from proliferations.

Structure of the thallus:

The thallus is 200-250 μ thick and in section shows a very large loose medulla and a comparatively thin cortex as in *H. poryphyroides* (Text-fig. 11). The cortex is three- to five-layered, the innermost cells being irregular to substellate. These cells range from 40-60 μ in length and 15-25 μ in width. The middle cortical layers consist of more rounded cells, the innermost cells being 15-20 μ in diameter and the cells of the peripheral layer being 5-7 μ in diameter. The cells of the surface layer (the outermost cortical layer) are 2·5-6 μ wide and almost isodiametric, or up to one and a half times as long as broad. The medullary filaments are

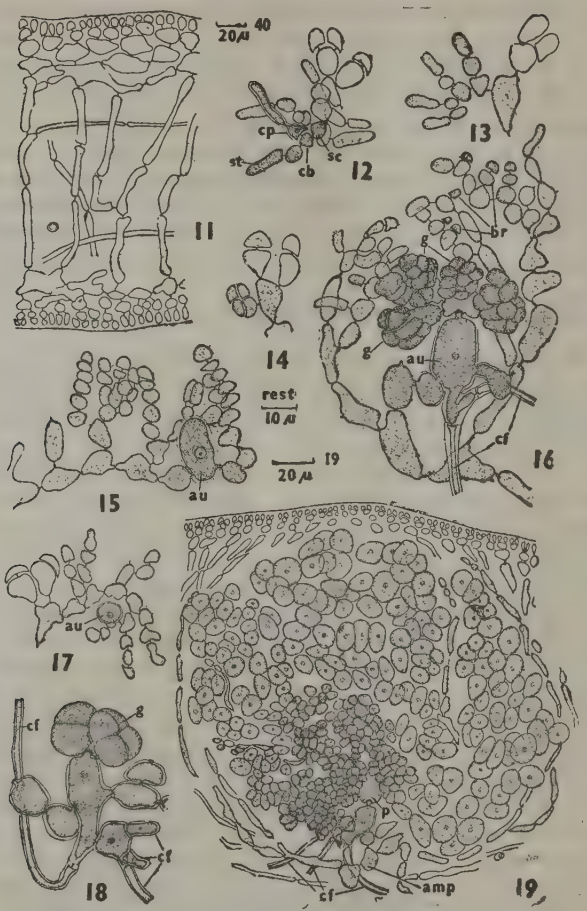
10-12 μ wide, simple or branched, and run transversely from cortex to cortex. Intermingled with the medullary filaments are slender thick-walled rhizoids 3-5 μ in thickness and stellate cells 10-25 μ in diameter and with arms up to 100 μ long. As in *H. porphyroides,* these stellate cells develop from the innermost cortical layer, later getting pushed into the medullary region. They are filled with a dense, deeply staining substance. Chromatophores are present in all cells including the medullary filaments, only the rhizoids and stellate cells lacking them. They are ribbon-shaped, but are, in general, shorter and narrower than in *H. porphyroides.*

Development of the carpogonial branches:

The carpogonial branches are developed in special ampullae which arise as accessory laterals from the cells of the middle cortex (Text-fig. 12). The primary ampullary filament is four to five cells long, the cells being subcylindrical to spherical and 5-6 μ in diameter. Short, two- to four- (rarely five-) celled erect secondary ampullary branches are developed from the cells of the primary ampullary filament. The basal cells of these erect branches are 3-4 μ wide and isodiametric, or up to four times as long as broad; the upper cells are subspherical, and 3-4·5 μ in diameter. The terminal cells are again elongate, and up to 15 μ long and 3-4·5 μ wide. The entire carpogonial ampulla is a small subglobular structure, 15-20 μ in diameter, and protrudes slightly into the medullary region. The supporting cell of the carpogonial branch is an intercalary cell of the primary ampullary filament (Text-fig. 12 *sc*). The carpogonial branch is two-celled; the carpogonial branch cell has a two- (rarely three-) celled lateral sterile branch (Text-fig. 12 *st*). Fully mature carpogonia were not seen. The carpogonia observed were short conical, 6-7 μ wide at the base and almost of the same height. The trichogyne is somewhat slender and 2-2·5 μ broad with a noticeable basal constriction (Text-fig. 12). The primary ampullary filament is so curved that the carpogonium occupies the approximate centre of the ampulla.

Development of the auxiliary cells:

Like the carpogonial ampullae the auxiliary ampullae are accessory lateral branches of the middle cortical cells, but they are very much larger and more profusely branched than the carpogonial ampullae. In this species early stages in the development of the auxiliary ampullae could be followed very well (Text-figs.



TEXT-FIGS. 11-19: *Halymenia floresia* (Clem.) Ag.

11. Thallus in section showing the internal organization. 12. Carpogonial ampulla. 13, 14, & 17. Stages in the development of an auxiliary ampulla; note the subterminal position of the auxiliary cell (au) in fig. 17. 15. Fully developed auxiliary ampulla; note that the auxiliary cell is the subterminal cell of the primary ampullary filament. 16. Young gonimoblast; note the division of the gonimoblast into four gonimolobes; note also the side branchlets (br) issuing from the cells of the secondary ampullary branches. 18. A still earlier stage in gonimoblast development; note the formation of secondary connecting filaments from the cell at the base of the connecting filament continuing

13, 14, 17). The ampullary initial is cut off by a curved wall from the side of a cortical cell. By a series of longitudinal divisions, it develops into the primary ampullary filament; even in the early stages of development the primary ampullary filament is noticeably curved (Text-fig. 13). While the development of the primary ampullary filament is progressing, the older cells also initiate the secondary ampullary branches on their dorsal sides (Text-figs. 13, 14).

The primary ampullary filament is six to nine celled; the cells are subspherical when young, but with maturity the lowermost three or four cells become very long and slender, 5-10 μ wide and up to 30 μ long, while the upper three to five cells still remain subspherical or elongate very slightly to become subellipsoidal, measuring 16-15 μ long and 6-10 μ broad.

The erect secondary ampullary branches are either simple or branched and up to ten cells long. While young all cells of the secondary branches are subspherical; but in the older ampullae, the cells of the secondary branches also alter their shape. Only very rarely do all the cells of a secondary ampullary branch remain subspherical as they are when young. Usually, the lower two or three cells of a branch become much elongated, measuring 3-5 μ in width, and up to five times as long as broad while the upper ones remain subspherical and are 5-7 μ in diameter. The terminal cells of the secondary ampullary branches are slightly elongated, 7-8 μ long and 3-4 μ wide, with the tip blunt or rounded.

The auxiliary cell is almost always the subterminal cell of the primary ampullary filament (Text-figs. 15, 17); occasionally it is the cell just below it. It is prominent even when the ampullary cluster is quite young (Text-fig. 17 *au*). When fully mature, the auxiliary cell is oblong-rounded, 15-30 μ long and 10-18 μ broad, thickwalled, densely protoplasmic and uninucleate (Text-fig. 15). The curvature of the primary ampullary filament is quite

its growth after fusion. 19. Ripe cystocarp in section; note the persistent primary ampullary filament which still retains its curvature; note also the loose pericarp formed by the secondary ampullary branches, and the prominent pedicel cell.

(*au*: auxiliary cell; *amp*: primary ampullary filament persisting in the ripe cystocarp; *br*: side branchlets developing from the secondary ampullary branches; *cb*: carpogonial branch cell; *cf*. connecting filament; *cp*: carpogonium; *g*: gonimolobes; *gi*: gonimoblast initial; *p*: pedicel cell; *sc*: supporting cell; *st*: lateral sterile branch).

pronounced in this species (Text-fig. 17), probably due to the fact that the auxiliary cell is situated very near the tip of the filament. Due to this curvature the auxiliary cell comes to occupy the centre of the ampullary cluster. The mature auxiliary ampulla is shallower than in *H. porphyroides,* being more like a bowl than a conical flask. In addition, mainly owing to the fact that the lower cells of the secondary ampullary branches become long and slender while the upper ones remain subspherical, the mature auxiliary ampulla in *H.floresia* is markedly different in aspect from that of *H. porphyroides.* The auxiliary cell in *H. floresia* is also slightly smaller than in *H. porphyroides.*

Post-fertilization development:

The connecting filaments are thick-walled, nonseptate and 2·5-3·5 μ in width. These are seen running in all directions in the cortex and outer medullary region, seeking out the auxiliary cells. As in *H. porphyroides,* there is a swelling of the connecting filament at the point of fusion with an auxiliary cell; in most instances the connecting filament continues its growth from this bulged out portion. Just as in *H. porphyroides,* here also a short cell is cut off at the base of the connecting filament; this cell enlarges and in its turn sends out several connecting filaments (Text-fig. 18 *cf*) which persist for quite a long time and can be seen in ripe cystocarps as a rhizoid-like cluster at the base (Text-fig. 19).

The gonimoblast initial by continued activity gives rise to several gonimolobes (Text-fig. 16 *g*) which ultimately develop into a compact subglobose gonimoblast in which the outlines of the constituent gonimolobes are indistinguishable. The lower derivative of the auxiliary cell enlarges considerably during gonimoblast development, but it still retains its original shape (Text-fig. 16 *au*). The primary ampullary filament also persists in a more or less intact condition, and can be clearly made out even in ripe cystocarps (Text-fig. 19 *amp*), although the secondary ampullary branches often get crushed beyond recognition by the developing gonimoblast. The protoplasmic connections between the auxiliary cell and the adjacent cells of the persistent primary ampullary filament widen, evidently for nutritive purposes (Text-figs. 16, 18).

As in *H.porphyroides,* here also very short lateral branches are developed from the erect secondary ampullary branches during

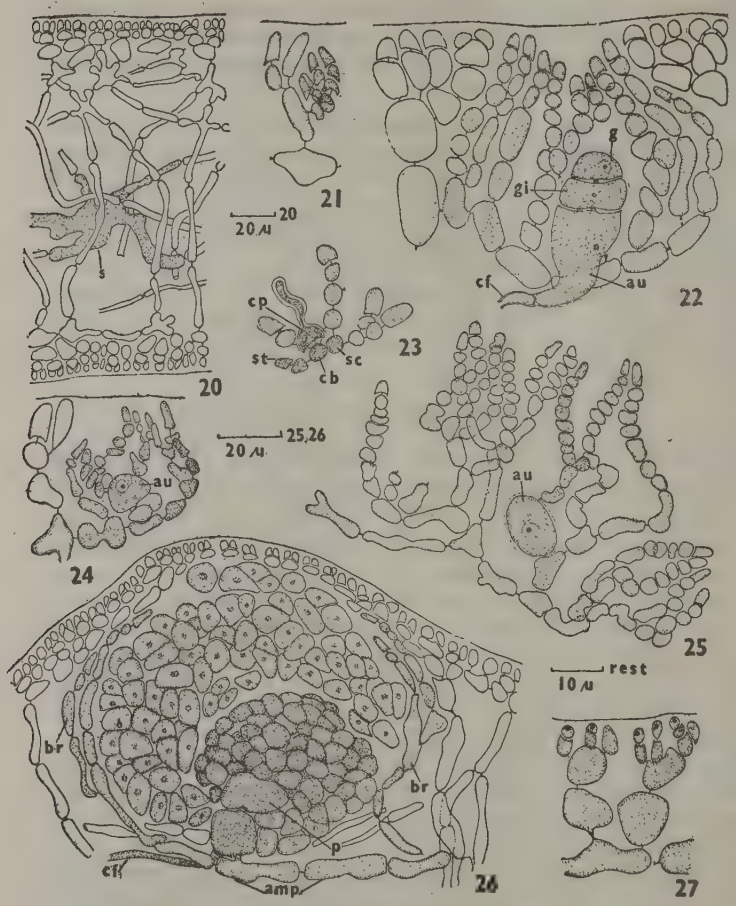
gonimoblast development. These branches are initiated approximately at the same time as the formation of the gonimoblast initial; they are not developed to the same extent as in *Grateloupia*. The entire ampulla with its short branchlets persists, forming the cystocarp wall.

The ripe cystocarps are scattered all over the thallus surface. They are subspherical, about 300 μ in diameter and fully immersed in the thallus, occupying most of the medulla in the region of their production. The enlarged lower derivative of the auxiliary cell (the foot) and the 'pedicel cell' are prominent features of the cystocarp (Text-fig. 19). The cystocarps have a central ostiole through which the ripe carpospores escape. The ripe carpospores are irregularly rounded, 10-15 μ in diameter (rarely a little more), uninucleate, densely protoplasmic and contain a number of short ribbon-shaped chromatophores.

HALYMENIA DILATATA ZANARDINI

Material: Pamban Bridge, 3rd April, 1938. *leg.* M.O.P. Iyengar.

Halymenia dilatata was first described by Zanardini (1851) based on a specimen from the Red Sea. It is now known to have a wide distribution, having been reported from the Somaliland Coast, the Malayan Archipelago, the coast of Japan and from Viet Nam. From the Indian region it was first reported by Boergesen (1938) from Malvan on the Bombay coast based on a cystocarpic specimen collected by Dixit and sent to him. Boergesen (*loc. cit.*, p. 214) says that these specimens agreed very well with Zanardini's description except for the maculae. Boergesen also makes mention of a maculate tetrasporic specimen collected by Edgar Thurston from the Madras Beach in 1900 and deposited in the British Museum herbarium. Boergesen referred this specimen also to *H.dilatata* but with some doubt as it showed some anatomical differences from the Malvan specimens. Dixit (1940) subsequently reported the alga from Malvan Harbour and said that the plants collected by him were very similar to those described by Boergesen earlier. Prof. Iyengar's collections of this species from Pamban included several well developed cystocarpic specimens which showed maculation of the thallus. A small cystocarpic specimen, however, was not maculate, but otherwise agreed with the maculate specimens in general features. All specimens in the Iyengar collection agreed well with the descriptions of this species given by Okamura (1921) from Japan and Dawson (1954 b) from Viet Nam.



TEXT-FIGS. 20-27: *Halymenia dilatata* Zanard.

20. Thallus in section showing internal organization. 21. Young ampullary cluster arising as an accessory lateral branch from a cortical filament. 22. Auxiliary ampulla showing an early stage in gonimoblast development. 23. Carpogonial ampulla. 24. Young auxiliary ampulla; note that the auxiliary cell is already conspicuous by its large size and dense contents. 25. Fully developed auxiliary ampulla; note that the auxiliary cell is the basal cell of a secondary ampullary branch. 26. Nearly mature cystocarp in section; note the persistent primary ampullary filament (amp) and the secondary ampullary branches (br)

The alga is attached to the substratum by a small discoid holdfast. The base of the blade is shortly cuneate, expanding rather abruptly into the wide foliaceous lamina; the blade is linear and divided into a varying number of rounded lobes which are pronouncedly undulate and imbricate, overlapping considerably. The blade is much mottled, the patches being irregular in shape and resembling sori in appearance. The laminae are up to 30 cm. long and 5-12 cm. wide; the margins are very wavy and laciniate-dentate, with a large number of minute proliferations all along the edges.

Structure of the thallus:

The thallus is 150-200 μ (rarely more) thick. In section it shows the typical internal organization of *Halymenia* (Text-fig. 20). The medulla is large and loosely constructed, with numerous anticlinally placed simple or branched medullary filaments 6-10 μ wide running from cortex to cortex, at times somewhat obliquely. Stellate cells are better developed and more numerous in *H.dilatata* than in the other two species described earlier. The stellate cells are 30-35 μ in diameter and with arms up to 150 μ long. The majority of them get filled with a dense, deeply staining substance in older thalli (Text-fig. 20 *s*). Running longitudinally in the medullary region are slender thick-walled rhizoids 3-5 μ wide. These often connect the stellate cells with one another or with the branches of the medullary filaments.

The cortex is thin, three- to five-layered, with the innermost cells substellate. The medullary filaments are connected with the arms of these cells. The cells of the middle cortical layers are irregular rounded, 8-15 μ long and 6-10μ broad, and get progressively smaller as we approach the periphery. The outermost layer of the cortex is composed of short cylindrical rounded cells 3-6 μ wide and as long as broad or occasionally slightly longer. The cells of this superficial layer are well pigmented, the chromatophores being discoid to ribbon-shaped. As in *H.porphyroides* and *H.floresia*, only the stellate cells and the rhizoids in the medullary region lack chromatophores. But in general, the chromato-

forming the pericarp. 27. Part of thallus in section showing spermatangia.

(*amp*: persistent primary ampullary filament in the ripe cystocarp; *au*: auxiliary cell; *br*: persistent ampullary branches forming a pericarp; *cb*: carpogonial branch cell; *cf*. connecting filament; *cp*: carpogonium; *g*: gonimoblast; *gi*: gonimoblast initial; *st*: lateral sterile branch; *s*: stellate cell; *sc*: supporting cell).

phores are fewer in number and more elongated in the cells of the interior.

Development of spermatangia:

The plants are monoecious. The spermatangia are aggregated in irregular sori, which appear as patches, in the younger portions. The spermatangial-mother-cells are cells of the outermost cortical layer in the fertile region. These cells are more elongated than normal cortical cells and produce one or two spermatangia terminally (Text-fig. 27). The spermatangia are ovoid to subspherical and 2-2·5 (3) μ in diameter. They show the typical organisation of the Floridean spermatangium. The nucleus occupies the upper part, close to the wall, appearing suspended above the large posterior vacuole. The cytoplasm forms a thin lining to the wall of the spermatangium (Text-fig. 27).

Development of the carpogonial branches:

The carpogonial branches are formed in rather small ampullae which are accessory laterals of the middle cortical cells (Text-fig. 23). The primary ampullary filament is three- to five-celled, the cells being sub-cylindrical to spherical and 3-5 μ in diameter. From the cells of the primary ampullary filament four- to six-celled simple secondary ampullary branches arise. The cells of these secondary branches resemble those of the primary ampullary filament and are 3-5 μ wide, cylindrical and isodiametric or subspherical. The terminal cells of the secondary ampullary branches are rather short as in *H.porphyroides*, cylindrical with the tips rounded, about 4 μ wide and up to 7 μ long. The supporting cell of the carpogonial branch is an intercalary cell of the primary ampullary filament (Text-fig. 23 *sc*). The carpogonial branch is two-celled, consisting of the carpogonium and the carpogonial branch cell. The carpogonium is subhemispherical with the trichogyne growing out from the centre of the rounded top. The trichogyne is constricted at the base. The carpogonial branch cell has a short two- to three-celled lateral sterile branch (Text-fig. 23 *st*). Fully developed carpogonial ampullae were not seen.

Development of the auxiliary cells:

The auxiliary cell ampullae, like the carpogonial ampullae, are also secondary laterals of the middle cortical cells (Text-figs. 21, 24). They are, however, very much larger and more profusely branched. The primary ampullary filament is six to nine cells long. The cells are subspherical in the younger stages, but later become

cylindrical, and 6-8 μ wide and 10-15 μ long, the lower three being often up to 30 μ long. The primary ampullary filament is coiled as in the two previously described species. Erect secondary ampullary branches are produced from all cells of the primary ampullary filament. These erect branches are slightly curved, simple or branched, and up to twelve cells long. In the young ampullae the cells of the secondary ampullary branches are subspherical, the terminal cells alone being subconical. In fully mature ampullae, however, the lower cells of the branches are cylindrical and up to 15 μ long and 5-6 μ wide; the upper ones remain subspherical and are 5-6 μ in diameter (Text-fig. 25). Only occasionally do we meet with mature auxiliary ampullae in which the lower cells of the secondary erect branch are also spherical. The terminal cells of the secondary ampullary branches are subconical or cylindrical with a tapering tip, and are 9 μ long and 3 μ wide. The mature ampullary cluster is 60-80 μ wide at the base, narrowing to 30-45 μ at the top. It is conical flask-shaped as in *H. porphyroides,* but with convex sides. As the secondary ampullary branches are curved and with rather blunt ends, the entire ampulla has a different aspect from those of *H. porphyroides* and *H. floresia.*

The auxiliary cell is intercalary, normally the basal cell of a secondary ampullary branch. Only occasionally does a cell of the primary ampullary filament function as the auxiliary cell. It is easily distinguished even in early stages by its larger size and denser contents (Text-fig. 24 *au*). In the fully mature ampulla, the auxiliary cell is very much larger than the rest of the ampullary cells, ovoid to subspherical and 15-23 μ long and 12-20 μ wide. It is densely protoplasmic and uninucleate; the wall, however, is not so thick as in the other two species described here (Text-fig. 25 *au*).

Post-fertilization development:

Post-fertilization stages in the carpogonial ampulla were not observed but connecting filaments were seen running through the cortical and outer medullary regions and fusing with auxiliary cells. These connecting filaments are non-septate, thick-walled and 2-2·5 μ in diameter. The connecting filament bulges out only slightly at the point of fusion with the auxiliary cell (Text-fig. 22), and usually there is no continuation of its growth as in *H. porphyroides* or *H. floresia.* Occasionally, however, a single new connecting filament may grow out of this bulged out portion, seeking out other auxiliary cells. In one or two isolated instances a short

cell was formed at the base of the new connecting filament; from this, a few more connecting filaments were produced.

After fusing with the connecting filament, the auxiliary cell enlarges and cuts off the gonimoblast initial at the top by a curved wall (Text-fig. 22 *gi*). The gonimoblast initial gives rise to the gonimoblast, which, when fully mature, is a subglobose compact mass, in which the outlines of the individual gonimolobes are obscured. The lower derivative of the auxiliary cell continues to enlarge during gonimoblast development. The protoplasmic connections between it and the adjacent cells of the primary ampullary filament enlarge to some extent, but open communication is never established. The lower derivative of the auxiliary cell and the enlarged gonimoblast initial are prominently seen in the ripe cystocarps as the foot and pedicel cells (Text-fig. 26).

The formation of lateral branchlets from the secondary ampullary branches after fertilization is a little more pronounced here than in *H. porphyroides* or *H. floresia*. These branchlets are initiated just at the time of formation of the gonimoblast initial, occasionally even earlier. The branchlets anastomose slightly forming a loose basket-like structure. The entire ampulla with these branches persists as the pericarp (Text-fig. 26 *br*.). The ripe cystocarps are scattered, subspherical to slightly flattened, 100-175 μ in diameter and 100-150 μ high. They are fully immersed in the thallus, projecting to a considerable extent into the medulla. There is a central ostiole at the top through which ripe carpospores escape. Ripe carpospores are rounded, uninucleate, 10-15 μ in diameter, and contain a number of ribbon-like chromatophores.

Weber van Bosse (1921, p. 237) makes a few remarks about gonimoblast development in this species. She says that though it was rather difficult to follow the stages clearly on account of the compact nature of the cortex, there seemed to be general agreement with what she had already observed in *H. durvillaei* (Weber v. Bosse, *l.c.* p. 233). The details of post-fertilization development described here are in general agreement with what she has described for *H. durvillaei*.

HALYMENIA POLYDACTYLA BOERGESEN

Material: Okha, 6th Jauary, 1955, *leg.* T. V. Desikachary.

Halymenia polydactyla was first described by Boergesen in 1932 and is based on specimens collected from Okha Port and

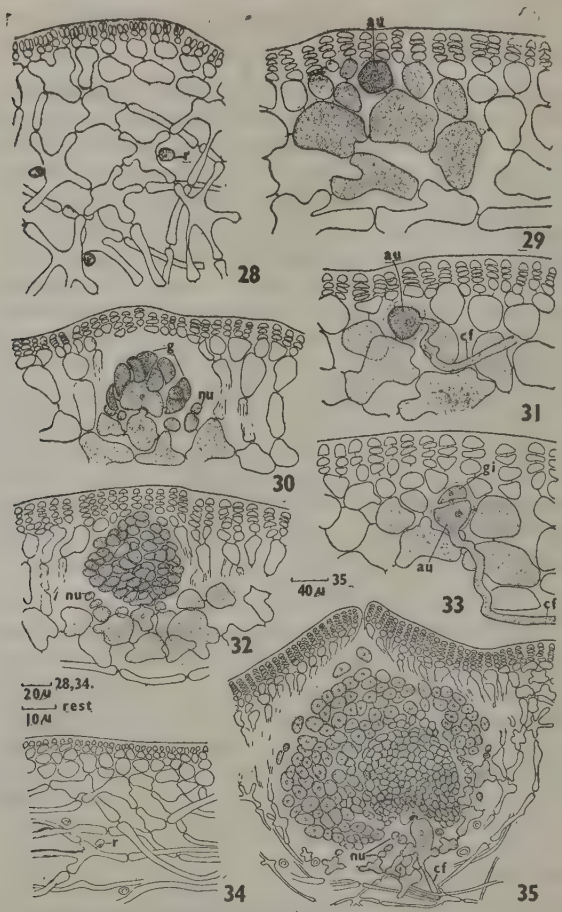
Dwarka. The material used in the present investigation was collected from the type locality.

The alga grows attached to rocks facing the open sea. The plants are rather large, reaching up to 27 cm. in height. Attachment to the substraum is by means of a small discoid holdfast 2-4 mm. wide. From this basal disc the erect frond arises. The frond is repeatedly forked, fastigiate, but with a tendency to become flabellate (Plate VI, Fig. 2). The lowermost undivided segment is cuneate, short, robust and compressed, the distance between the base and the first forking being about 2 cm. The intervals between successive dichotomies is variable; in the basal part it is a little less than 2 cm. in the upper portions up to 5 cm. The segments are terete or a little compressed below, cylindrical above and about 5-7 cm. thick. The width just below the forkings is about 15 mm. The axils are noticeably rounded. Occasionally, proliferations are also produced from the lower segments (Plate VI, Fig. 1). These proliferations are quite similar to the main frond in their general configuration. The apices of the uppermost segments are blunt to rounded. The plants are dark purple in colour in the basal parts, the colour grading to paler red in the upper portions. In consistency they are elastic and tough.

Boergesen (1932) had only tetrasporic specimens. Fine fully grown sexual specimens are present in Desikachary's collection. The sexual plants are similar to the tetrasporic plants in appearance. The cystocarps are scattered on the older portions of the thallus, only the apices being free; they appear as pinkish dots and are just visible when the plants are held up against bright light. The cystocarps are fully immersed in the thallus, only the central ostiolate portion jutting out as a minute conical projection.

Structure of the thallus:

The thallus is multiaxial in construction, with growth of the fountain type. A section shows that the medulla is very large in comparison to the cortex (Text-figs. 28, 34). The cortex is four to seven layered; the outermost layer is quite compact and consists of small, thick-walled, and densely pigmented cylindrical cells, 6-9 μ in diameter and 3-11 μ in height. In surface view, these cells appear subpolygonal on account of mutual compression. Cells of the middle cortex are a little larger and more loosely arranged. The two innermost cortical layers consist of very large loosely packed irregularly lobed cells 20-35 μ in diameter. These cells



TEXT-FIGS. 28-35: *Sebdenia polydactyla* (Boergs.) Balakrishnan

28. Transverse section of thallus showing internal organization; note the rhizoid initials (r). 29. Longitudinal section of fertile portion of the thallus showing an auxiliary cell before fertilization; note that the auxiliary cell (au) is an intercalary cell of a normal vegetative filament; note also that the cortical cells in the neighbourhood of the auxiliary cell are filled with dense contents. 30. Early stage in gonimoblast development and formation of the cystocarp; note the short nutritive branchlets (nu) arising from the cortical cells surrounding the

EXPLANATION OF PLATE VI. FIGS. 1-4.

1, 2 *Sebdenia polydactyla* (Boergs.) Balakrishnan. Tetrapetype tetrasporic specimens collected by Desikachary from Okha in January 1955. Note the proliferations issuing from the basal portion of the specimen in FIG. 1. 3 *Halymenia porphyroides* Boergs. Tetrasporic material from Okha collected by Desikachary in January 1955. 4 *Halymenia floresia* (Clem.) Ag. Specimen No. 191 Collected by Iyengar from Chank Fisheries Beds, Rameshwaram, in September 1934.

grade into the stellate cells of the outer medulla which are 20-25 μ in diameter and short-rayed, the arms being only up to 50 μ long. As we progress towards the inner medulla the rays of the stellate cells get longer and longer. The very loose central medulla is composed of longitudinal filaments with cells 4-20 μ wide and up to 130 μ long. The medullary filaments are intermingled with thick walled, multicellular unbranched rhizoids 3-7 μ thick, and the stellate cells which are pronouncedly ganglioid. The stellate cells of the outer medulla often bear short spherical or rounded cylindrical cells which are filled with dense contents (Text-figs. 28, 34 *r*). Boergesen (1932, p. 123) calls these cells 'gland cells' but they appear to be rhizoid initials. Numerous cases were seen in which they gave rise to the slender thick-walled rhizoidal filaments which lie intermingled with the medullary filaments in the central region. Chromatophores are present in all cells of the cortex and outer medulla. They are disc-shaped to ribbon-shaped, the number and intensity of pigmentation decreasing as we go to the interior.

Development of spermatangia :

The sexual plants are monoecious. The spermatangia occur in dense sori which form large patches just below the tips. The sori extend almost throughout the entire circumference and by their coalescence form a fertile band just in advance of the cystocarpic zone. The spermatangial-mother-cells are elongated and each one bears at the top one or two spermatangia (Text-fig. 36). The spermatangia are elongate ovoid, 3 μ × 2-2·5 μ, and show the typical organization of the Floridean spermatangium, with a posterior vacuole, the nucleus adpressed to the wall at the top and the cytoplasm forming a thin lining to the spermatangium (Text-fig. 36).

auxiliary cell; note also the rupture of the middle cortical cells due to the enlargment of the gonimoblast. 31. A connecting filament fusing with the auxiliary cell. 32. A more advanced stage than that shown in fig. 30, note the well developed gonimoblast and the plexus of nutritive cells surrounding the auxiliary cell; note also the rupture of the cortical cells to form the cystocarpic cavity. 33. Auxiliary cell cutting off the gonimoblast initial (gi) after fusion with a connecting filament. 34. Longitudinal section of thallus. 35. Ripe cystocarp in section.

(*au*: auxiliary cell; *cf*: connecting filament; *g*: gonimoblast; *gi*: gonimoblast initial; *nu*: branchlets formed by the cells of the nutritive plexus; *r*: rhizoid initial.)

Development of the carpogonial branches:

The carpogonial branches are not formed in special ampullary clusters as in the three species of *Halymenia* previously described in this paper. They arise directly from a cell of the middle cortical layers. In other words, the supporting cell of the carpogonial branch is not an intercalary cell in a specially formed accessory branch system, but an intercalary cell of a normal vegetative filament (Text-figs. 37, 39 *sc*). The carpogonial branch is three to four-celled and simple; the lower sterile cells of the carpogonial branch (the carpogonial branch cells) do not have side branches (Text-figs. 37, 39). The carpogonium is hemispherical, with a slender trichogyne of variable length. The carpogonial branch cells are subspherical and 6-8μ in diameter; generally, the lowermost is the broadest and there is a slight tapering towards the carpogonium. The carpogonium itself is 5-7μ wide at the base and up to 10μ high; the trichogyne is 1-3μ wide and at times winds tortuously through the cortical cells before its emergence (Text-fig. 39). Usually, the supporting cell bears only one carpogonial branch (Text-fig. 37). Occasionally, however, it may have two (Text-fig. 39), or even three.

Development of the auxiliary cells:

The auxiliary cells also are not formed in special ampullae or accessory branch systems. The auxiliary cell is a cell in one of the middle cortical layers, remote from the carpogonial branch. That is to say, like the supporting cell of the carpogonial branch, the auxiliary cell is an intercalary cell of a normal vegetative filament (Text-fig. 29), and not an intercalary cell in a specially developed accessory branch system as in the other three species of *Halymenia* investigated. The auxiliary cell is uninucleate, densely protoplasmic and quite conspicuous even before fertilization (Text-fig. 29 *au*). The fully developed auxiliary cell is subspherical, at times a little angular due to the pressure of the surrounding cells and 10-15μ in diameter. The cortical cells in the neighbourhood of the auxiliary cell also are usually filled with somewhat dense contents and are conspicuous (Text-fig. 29). The wall of the auxiliary cell is slightly thicker than that of the surrounding cortical cells.

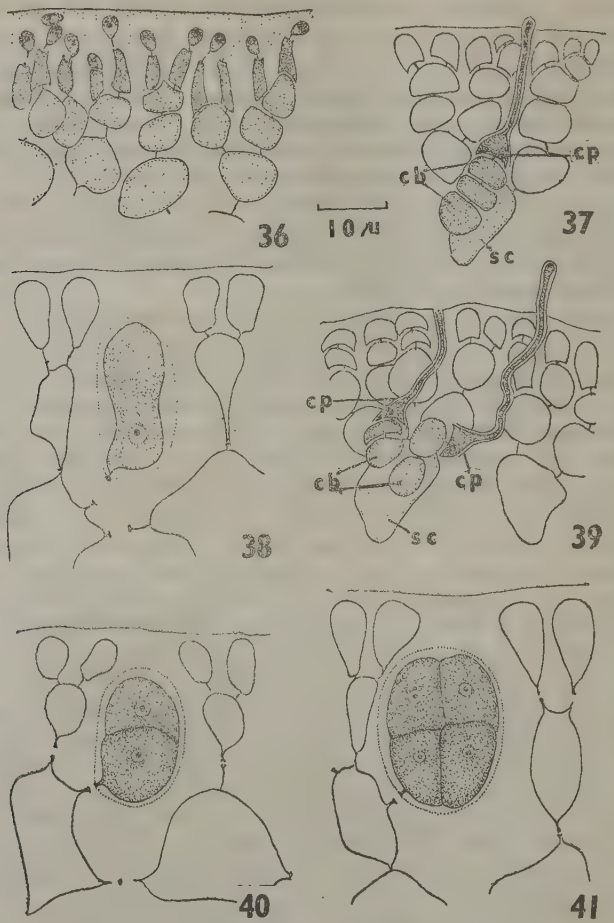
Post-fertilization development:

Post-fertilization stages in the carpogonial branches were not observed. Non-septate, thick-walled, densely protoplasmic connecting filaments, 3-4μ wide, were seen running through the middle

cortex in all directions. These seek out the auxiliary cells and fuse with them (Text-fig. 31). After this fusion, and presumably the transfer of a diploid nucleus from the connecting filament, the auxiliary cell cuts off the gonimoblast initial on the top (towards the thallus surface) by a slightly curved wall (Text-fig. 33 *gi*). By further division, the gonimoblast initial produces the compact gonimoblast cluster. The lower derivative of the auxiliary cell also enlarges during gonimoblast development and forms a sort of pedicel at the base of the gonimoblast. The connecting filament was never seen to continue its growth after fusing with an auxiliary cell (Text-figs. 31, 33, 35, *cf*).

Approximately at the time of fusion of a connecting filament with the auxiliary cell (at times even earlier) the cells of the surface cortical layer start dividing transversely (Text-figs. 29, 31, 33). As a result of a number of such divisions, the portion of the cortex above the fertilized auxiliary cell develops into a sort of an arched wall consisting of four to five layers of subdiscoid cells, immediately overlying the developing gonimoblast (Text-figs. 30, 32). However, there is no division of the cells of the middle cortex surrounding the auxiliary cell to keep pace with gonimoblast development. Finally, owing to the pressure of the developing gonimoblast, the cells of the middle cortex get raptured, giving rise to a vault like cystocarpic cavity, the centre of which is occupied by the developing gonimoblast (Text-figs. 30, 32). During later stages, the four to five layered wall-like portion lying above the developing gonimoblast gradually gets pushed up more and more till it finally develops into a conical protuberance, pierced in the centre by the ostiole (Text-fig. 35). In *H. polydactyla*, therefore, the pericarp or cystocarp wall is developed after fertilization, by the division of the outer cortical cells. The wall, however, is confined only to the upper portion of the cystocarp, forming, as it were, an arched roof to the gonimoblast. The gonimoblast itself is not surrounded by an envelope, the cystocarpic cavity being formed by the mere rupture of the cortex. Development of a definite pericarp by post-fertilization divisions in the outer cortex is a feature not seen in the other three species of *Halymenia* studied.

As already stated, the cortical cells in the immediate vicinity of the auxiliary cell are densely protoplasmic (Text-figs. 29, 31, 33); these cells enlarge considerably during gonimoblast development. Generally, they produce short branches, the cells of which are densely protoplasmic. (Text-figs. 30, 32, *nu*). These large cells, which occupy the floor of the cystocarpic cavity surrounding the



TEXT-FIGS. 36-41: *Sebdenia polydactyla* (Boergs.) Balakrishnan.

36. Transverse section of a young portion of the thallus showing the formation of spermatangia. 37. Carpogonial branch, arising from an intercalary cell of a cortical filament. 38. Young tetrasporangium. 39. Two carpogonial branches arising from the same supporting cell. 40. Tetrasporangium after the first division. 41. Ripe tetrasporangium, with cruciately arranged tetraspores.

(*cb*: carpogonial branch; *cp*: carpogonium; *sc*: supporting cell.)

much enlarged lower derivative of the auxiliary cell, constitute a nutritive tissue furnishing nourishment to the developing gonimoblast. There is a gradual impoverishment of these cells as also the cells of the short branches produced by them. While the cells themselves persist, the majority of the branches produced by them apparently get used up and remnants of them can be distinguished in the ripe cystocarps (Text-fig. 35 *nu*). In this feature also *H. polydactyla* differs from the other three species of *Halymenia* described earlier.

The ripe cystocarps are subspherical, about 200μ in diameter, not totally sunk in the thallus, the top portion projecting as a conical protuberance with a central ostiole (Text-fig. 35). The carpospores are subspherical, 15 to 20μ in diameter, uninucleate and contain a number of small ribbon-shaped chromatophores.

Development of tetrasporangia:

The tetrasporangia are one-celled lateral branches of cells of the outer cortex. The tetraporangium initial is cut off by a slightly curved wall from the top of the mother cell. It soon enlarges considerably in size, becoming somewhat oblong-rounded (Text-fig. 38) and then divides transversely into two (Text-fig. 40). Each one of the daughter protoplasts divides once more vertically so that four tetraspores are formed; the tetraspores are cruciately or decussately arranged (Text-fig. 41). Occasionally, the division planes are a little oblique so that the tetraspores are irregular in arrangement. The tetraspores are uninucleate, densely protoplasmic and possess a number of ribbon-shaped chromatophores. The ripe tetrasporangia are oblong-ellipsoidal and 20-27 μ long and 19-24μ wide.

In the details of pre- and post-fertilization development the first three species of *Halymenia* described here, *viz., H. porphyroides, H.floresia* and *H.dilatata,* show general agreement. With a few minor differences, they exhibit a uniform pattern, which can be summarised as follows:

The carpogonial branches and auxiliary cells are formed in special ampullae which are accessory branch systems. The supporting cell of the carpogonial branch is an intercalary cell of the primary ampullary filament. The carpogonial branch is two-celled, and the carpogonial branch cell has a short two to four-celled lateral sterile branch. After fertilization, the carpogonium enlarges and, without fusion with any cell or cells of the carpogonial ampulla, sends out connecting filaments, which fuse with auxiliary cells.

After fusion with a connecting filament, the auxiliary cell cuts off the gonimoblast initial at the top towards the surface of the thallus. The gonimoblast initial then develops into a compact gonimoblast. The connecting filament, after fusion with an auxiliary cell, is capable of continuing its growth and fusing with more auxiliary cells. Very often, a short cell is formed at the base of this continuation and from this a number of secondary connecting filaments arise. The ampullary cluster, along with the short lateral branches produced after fertilization, persists in the ripe cystocarp forming the cystocarp wall.

The details of pre- and post-fertilization development in *H.polydactyla,* however, are totally different from that of the other three species considered above. The main points of difference are:

1. The supporting cells of the carpogonial branches and the auxiliary cells are not produced in special ampullae, or accessory branch systems; on the contrary, they are intercalary cells of normal vegetative cortical filaments.

2. The carpogonial branches are three to four-celled, not two-celled, and the carpogonial branches do not have side branches.

3. The cystocarp has an arched wall at the top, which is formed by the post-fertilization activity of the surface cortical cells in the region, and is not completely immersed in the thallus as in the other three species.

4. A special nutritive tissue of very large irregular cells is developed from the cortical cells which lie round the auxiliary cell; this tissue persists in the ripe cystocarp forming a prominent basal plexus surrounding the lower derivative of the auxiliary cell.

All these differences indicate that *H.polydactyla* is not a *Halymenia* at all and that its affinities have to be sought elsewhere,

The method of initiation of the carpogonial and auxiliary cell branches is an important feature in separating the two orders Cryptonemiales and Gigartinales, which are considered by many workers to be very closely related. These structures are accessory and are specially formed for the purpose in the Cryptonemiales, whereas in the Gigartinales the carpogonial branch initial and the auxiliary cell are already existent vegetative cells which become modified for purposes of reproduction. In *H.polydactyla* the carpogonial branch initial and the auxiliary cell are truly modified intercalary vegetative cells and not cells in an accessory branch system as is characteristic of the Cryptonemiales. Judged by this

character, *H.polydactyla* should be transferred to the Gigartinales. In the Gigartinales, it shows close affinity to the families Nemastomaceae and Sebdeniaceae. In both these families, the thallus is multiaxial, the tetrasporangia are cruciately divided, and the carpogonia and auxiliary cells are remote, fusion between them being accomplished by connecting filaments.

H.polydactyla resembles genera of the Nemastomaceae in some of its vegetative features, in the structure and origin of the carpogonial branch and also in the gonimoblast being outwardly directed. But it differs from the members of the Nemastomaceae in the following features.

a. Stellate cells are absent in the Nemastomaceae whereas they are present in *H.polydactyla.*

b. The cystocarps of the Nemastomaceae are without pericarps, whereas the cystocarp of *H.polydactyla* has a definite pericarp.

c. After fusion with one auxiliary cell, the connecting filament in the Nemastomaceae is capable of continuing growth and fusing with more auxiliary cells whereas in *H.polydactyla* the connecting filament never continues growth after fusion with an auxiliary cell.

Because of these differences, *H.polydactyla* cannot be placed in the Nemastomaceae.

On the other hand, *H.polydactyla* shows very great resemblance to *Sebdenia,* the only genus of the Sebdeniaceae. Stellate cells are present in both species at present included under the latter genus (see Kylin, 1956, p. 250). There is great resemblance in other features of vegetative organization also. Further, the details of reproduction in *H.polydactyla* agree with those of *Sebdenia* (for *S. dichotoma,* see Berthold, 1884; for *S.monardiana,* see Sjöstedt, 1926). According to Sjöstedt (*loc. cit.*) the gonimoblast initial is cut off towards the thallus surface and is directed outwards (see also Berthold, *loc.cit.,* Pl. 8, fig. 9). In *Sebdenia* the cortical cells in the neighbourhood of the auxiliary cells are filled with dense contents and develop into a nutritive tissue (the 'podium' of Berthold, 1884, p. 13) surrounding the lower derivative of the auxiliary cell. In *S.monardiana,* Sjöstedt has described the activity of the outer cortical cells after fertilization culminating in the formation of an arched wall to the cystocarp. In both species of *Sebdenia,* the cystocarpic cavity is formed by the rupture of the cortex as a result of the pressure exerted by the developing gonimoblast. Finally, Sjöstedt's figures (Sjöstedt, *loc. cit.,* figs. 13c, 14a)

clearly show that in *S.monardiana* the connecting filament does not continue its growth after fusing with an auxiliary cell. All the above features are shared by *H.polydactyla* as has been revealed by the present investigation.

The writer feels that the alga described by Boergesen (1932) as *Halymenia polydactyla* should, therefore, be transferred to the genus *Sebdenia* (as *Sebdenia polydactyla* (Boergs.) Balakrishnan = *Halymenia polydactyla* Boergesen, Kew Bulletin, 1932, p. 122, Pl. IV).*

DISCUSSION

The present investigation has revealed a close agreement between the two genera *Grateloupia* (see Balakrishnan, 1961a) and *Halymenia*. The stages in the development of the reproductive structures before and after fertilization in the two genera follow a uniform pattern and these have been summarised in Balakrishnan (1960, p. 86 *et. sq.*). The following salient points emerge out of the present investigation.

1. Pre-fertilization development.

(a) The carpogonial branches and the auxiliary cells are borne separately, and quite remote, in distinct ampullary clusters. These ampullary clusters (ampullae) are accessory lateral branches arising from cells of the inner cortex. Each ampullary cluster consists of a primary ampullary filament which is 4-8 celled. Almost all the cells of the primary ampullary filament give rise to erect secondary ampullary branches which are 4-10 celled. The ampulla is a subconical flask-shaped structure, with the carpogonial branch, or the auxiliary cell, as the case may be, occupying approximately the centre at the base.

(b) The carpogonial ampullae are unicarpogonial, i.e., each ampulla has only one carpogonial branch. The supporting cell of the carpogonial branch is an intercalary cell of the primary ampullary filament. The carpogonial branch is two-celled, consisting of the carpogonium and a single carpogonial-branch-cell. The carpogonial branch cell has a short lateral sterile branch of 2-6 cells.

(c) The auxiliary cell ampullae are generally much larger and more profusely branched than the carpogonial ampullae. The auxiliary cell is an intercalary cell of the primary ampullary fila-

*See Balakrishnan, 1960, p. 89.

ment or the lowermost cell of one of the erect secondary ampullary branches. There is only one auxiliary cell in each auxiliary ampulla.

2. Post-fertilization development.

(a) After fertilization, the trichogyne is cut off. The fertilized carpogonium enlarges considerably and often attains a lobed appearance; from the enlarged carpogonium are sent out a varying number of thick-walled nonseptate connecting filaments, which presumably carry derivatives of the diploid zygote-nucleus. There is no fusion of the fertilized carpogonium or the connecting filaments with any cell or cells of the carpogonial ampulla. The connecting filaments run in all directions, seeking out the auxiliary cells and fusing with them. Often, a connecting filament establishes connection with more than one auxiliary cell. Further development (production of the gonimoblast) takes place only in the auxiliary ampullae. The auxiliary cell, after fusing with the connecting filament, and presumably after receiving a diploid nucleus from it, enlarges and cuts off the gonimoblast initial by a curved wall near the top. By further division, the gonimoblast initial produces a gonimoblast made up of several distinct gonimolobes (*sensu* Schmitz, Kylin etc. *non* Drew).

Two kinds of cystocarp are seen:

1. Cystocarps with a definite wall (pericarp) made up of a loosely interwoven network of filaments, e.g., *Halymenia* and 2. cystocarps without such a wall, e.g., *Grateloupia.* The cystocarp wall, in the case of *Halymenia,* is made up of the persistent branches of the auxiliary cell ampulla. In *Grateloupia,* however, though branches are developed in the auxiliary cell ampulla during the early stages of cystocarp development, these are soon used up. In other words the cystocarps here appear to be without a wall because the branches wither away very soon, having served a nutritive purpose.

A more or less similar pattern of development has already been reported for several other members of the Cryptonemiaceae: *Grateloupia proteus, G.dichotoma* and *Halymenia floresia* (Berthold, 1884); *Prionitis lanceolata* and *Cryptonemia borealis* (Sjöstedt, 1926); *Grateloupia filicina* (Kylin, 1930); *G.lithophila* (Balakrishnan, 1949); *Halymenia bifida, H.abyssicola, Carpopeltis (Polyopes) bushiae, Cryptonemia angustata* and *Prionitis andersoniana* (Dawson, 1954 a); *Grateloupia turuturu, Pachymeniopsis*

lanceolata, *P.yendoi* and *Phyllymenia* (*Cyrtymenia*) *sparsa* (Kawabata, 1954a-57). For all these cases more or less complete information is available. The same pattern probably also exists in *Halymenia durvillaei* (Weber van Bosse, 1921), *Grateloupia lancifolia*, *Halymenia acuminata* and *Prionitis cornea* (Okamura, 1908, 1913), *Grateloupia schizophylla*, *G.hancockii* and *Prionitis acroidalea* (Dawson, 1954a).

The genera *Cryptonemia*, *Grateloupia*, *Prionitis*, *Halymenia*, *Carpopeltis* (incl. *Polyopes* in part), *Pachymeniopsis* and *Phyllymenia* (*Cyrtymenia*), therefore, form a very natural assemblage showing remarkable uniformity in the development of reproductive structures. The most important characteristics are:

1. The carpogonial branches and auxiliary cells are separate and in special accessory branch systems (the ampullae).

2. The carpogonial ampullae are unicarpogonial; the carpogonial branch is two-celled.

3. From the fertilized carpogonium the connecting filaments proceed (*directly*) to one or more auxiliary cells and fuse with them.

The last feature, *viz.*, fusion of the connecting filaments directly with the auxiliary cells (without nutritive fusions in the carpogonial branch system), is particularly emphasized by Kylin (1937, 1956) who uses it as a distinguishing character between the Cryptonemiaceae and other non-procarpic families of Cryptonemiales.

Full details of the reproduction in the genus *Halymenia* are given here for the first time and these show that *Halymenia* resembles other genera of the family Cryptonemiaceae so far studied. The writer agrees with Kylin that the chief character of the family Cryptonemiaceae, distinguishing it from other non-procarpic Cryptonemiales, is the fact that here neither the fertilized carpogonium nor the connecting filaments produced by it fuse with any cell in the carpogonial branch; on the contrary, the fertilized carpogonium gives out connecting filaments directly, and these straightaway seek out the auxiliary cells and fuse with them.

This uniform pattern of sexual development which has so far been found to be present in a very large number of species belonging to the family can, therefore, be taken as the characteristic feature of the family. The present investigation has also revealed

that one species, *Halymenia polydactyla* Boergs., does not agree, in pre- and post-fertilization development, with other members of the Cryptonemiaceae.

The auxiliary cell in *Halymenia polydactyla* is an intercalary cell of a normal vegetative filament, not one in an accessory fertile branch system. Similarly, the carpogonial branch initial is also an intercalary cell of a normal vegetative filament. Hence, *H.polydactyla* should rightly be placed in the Gigartinales and not in the Cryptonemiales. Accordingly, it has been removed from the genus *Halymenia* and the family Cryptonemiaceae and transferred to the Gigartinales as a species of *Sebdenia* (Sebdeniaceae).

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